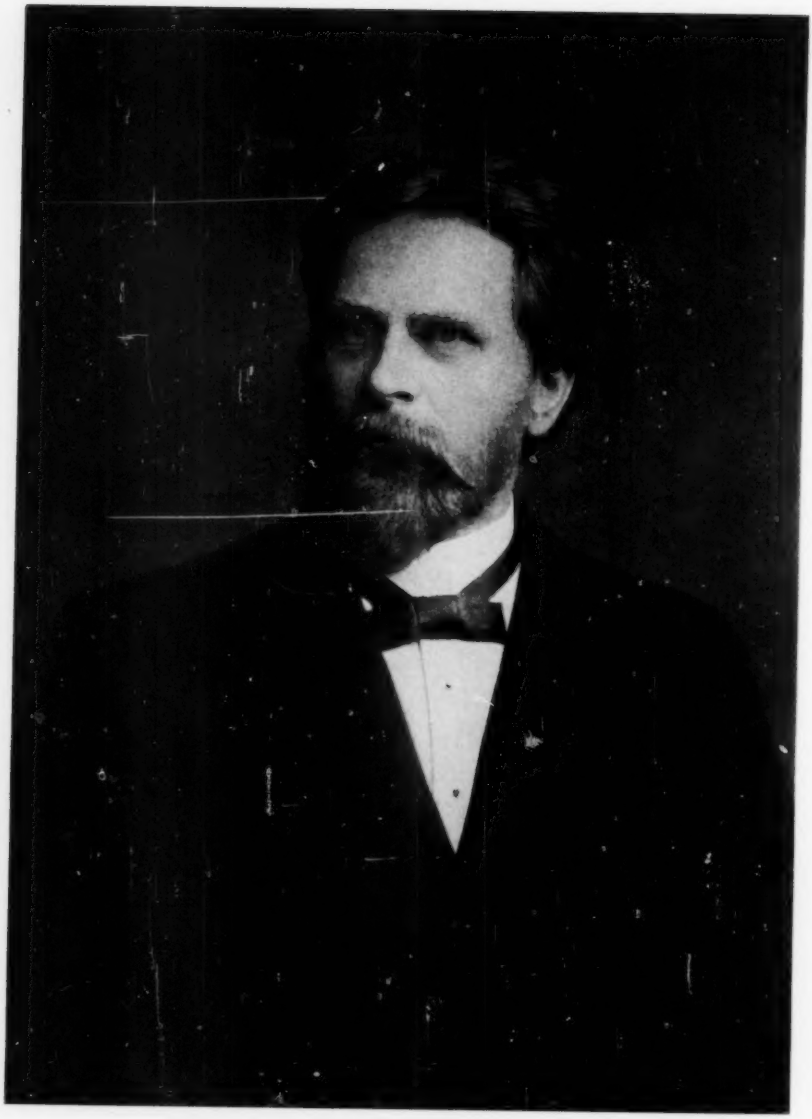
MAX RUBNER

(1854 – 1932)



MAX RUBNER

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(JUNE 2, 1854 — APRIL 27, 1932)

"Great men are very rare. They are worth knowing. They give impulse and stimulus to lesser men. They make the world more worth while for others to live in because of their presence in it. Max Rubner was the greatest man I ever knew." These are the words of Graham Lusk in a tribute to Rubner before the American Association for the Advancement of Science on June 23, 1932. One of the elements of Rubner's greatness was his ability to derive broad fundamental concepts from relatively simple experiments.

A native of Munich, Rubner started his scientific career as a pupil of Carl Voit in his early twenties. These were stirring times in the Munich laboratory. The new apparatus of Pettenkofer and Voit for accurately measuring the expired CO_2 of human subjects or experimental animals was rapidly changing the older theories of food metabolism. The major interest in Voit's laboratory at this time was centered around the study of energy transformations in the living body. From the determination of expired CO_2 and the carbon and nitrogen excreted in the urine, the energy metabolism of the fasting man was calculated in terms of grams of fat and protein oxidized in the body. Rubner's early contribution to the problem was the demonstration from bomb calorimeter determinations that about 25% of the total heat value of protein is lost to the body by the excretion of incompletely oxidized nitrogenous material in the urine and feces. His early data, published in 1879, are the basis for the present methods of calculating respiratory metabolism.

Voit had suggested a study of the interchangeability of fat and carbohydrate as sources of body energy. From experiments on fasting rabbits, Rubner in 1878 noted that protein

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could replace fat as a source of energy when the fat stores in the body were exhausted. Extending these investigations he conceived the isodynamic law, "that the food-stuffs may under given conditions replace each other in accordance with their heat-producing value." His standard values of 4.1 cal. per gram of protein, 9.3 cal. per gram of fat, and 4.1 cal. per gram of carbohydrate have had world-wide use in the calculation of the food and nutritional requirements of large populations.

With these new caloric values at hand, he began a recalculation of the existing data from metabolism determinations on man and on animals. Meeh had just published (1879) a simple formula for calculating surface area ($S.A. = KW^{2/3}$) substituting weight for volume, and introducing a constant (K) to correct for the varying body shapes from one species to another. The calculations showed that the 24-hour metabolism of Pettenkofer and Voit's fasting man per square meter of body surface was approximately equivalent to that of a man on a medium diet or of a fasting dog or a breast-fed infant.

This was the discovery of Rubner's "law of surface area," that the heat value of the metabolism of the resting individual is proportional to the area of the surface of his body. In my opinion it is his outstanding scientific achievement, both for its breadth of concept and its stimulating influence on research in metabolism, calorimetry, and nutrition. The stature of the concept may be measured by the fact that after more than half a century the subject is still a controversial issue.

During the period of these early investigations, a conflict of ideas developed between the great master and his youthful pupil. In 1881 Voit published his belief that "The unknown causes of metabolism are found in the cells of the organism. The mass of these cells and their power to decompose materials determine metabolism." Voit concluded from his studies of respiratory metabolism that there is an inherent rate of metabolic activity in the body cells which is augmented by the quantity and type of food material (protein, fat or carbo-

hydrate) brought to the cells in the blood stream. Environmental conditions or the need for energy are controlling factors but "cannot possibly be the cause of metabolism."

Influenced by his earlier work on the isodynamic law, Rubner was convinced that the study of energy changes in the body would produce the most rapid advances in basic knowledge of nutrition and metabolic processes. Despite Voit's dissension and the delay in publication until 1883, Rubner held firmly to his theory that the fundamental metabolism of a warm-blooded animal is always constant, and that the increased heat production after the ingestion of food is due to intermediary reactions superimposed on the fundamental level. Their opposing views were never reconciled.

The opportunity to produce experimental evidence for this theory came after he moved to Marburg in 1885 to become the first Professor of Hygiene, joining the notable company of Albrecht Kossel, Hans Horst Meyer, and Friedrich von Müller as a full-fledged independent investigator. Here in his own laboratory in 1889 he constructed, mainly by his own efforts, the first accurate respiration calorimeter. Among his initial experiments was the demonstration that the law of the conservation of energy applied to the living animal body, in that the heat loss from the body agreed with that calculated from the food materials oxidized. In a dog living in the calorimeter for 45 days the total calorimetric measurement of heat production was 17,349 cal. and that calculated from the respiratory metabolism and nitrogen excretion was 17,406 cal.

In 1891 Rubner was invited to Berlin to take Robert Koch's place as Professor of Hygiene. Then in 1909 he succeeded Engelman as Professor of Physiology, a position he occupied with distinction until he became emeritus in 1924. In the decade following the move to Berlin, the field of energy metabolism was vigorously explored; for example, 50 papers appeared in the Archives für Hygiene during this period. Studies of the agreement between the direct measurement of heat production and the respiratory metabolism were extended to

cover a variety of nutritive conditions and diets. A large amount of evidence was accumulated in support of the surface area law in mammals, ranging from the horse to the mouse. The effect of changes in environmental temperature on the metabolic rate was widely investigated, resulting in Rubner's well-known chart showing the areas of physical and chemical regulation of body temperature. Much effort was given to the search for an explanation of the extra heat production caused by the ingestion of food, particularly protein, a reaction which he called "specific dynamic action." The extensive experimental evidence related to these basic concepts which Rubner had obtained during this period was collected and published in 1902 in his comprehensive book, "Die Gesetze des Energieverbrauchs bei der Ernährung."

New interests in the following years continued to produce new concepts. He demonstrated that the energy from mechanical work during exposure to cold would replace the rise in metabolism (chemical regulation) found in the quiescent individual. Investigation of the energy requirements in growing animals led to the conclusion that "The amount of energy (calories) which is necessary to double the weight of the newborn of all species (except man) is the same per kilogram no matter whether the animals grows quickly or slowly." Attention was turned again to the sparing action of carbohydrate on protein metabolism. His eminent pupil, Karl Thomas, demonstrated in man with a starch-cream diet a minimum urinary nitrogen excretion of 2.2 gm daily, which Rubner designated as the minimum or "wear and tear" quota of protein metabolism. During World War I Rubner and his assistants were called upon by the German government to test a large variety of bread substitutes and modifications. Determinations of the fecal loss in nitrogen and calories in man and animals showed that none of the proposed changes gave a product nutritionally equal to white or rye bread.

To attempt to trace the impact of Rubner's pioneering work on the various fields of modern research involving energy metabolism would be a monumental task. Only a few out-

standing examples can be mentioned. The credit for the development of calorimetry in relation to respiratory metabolism undoubtedly is due to Rubner's foresight and persistence. Atwater, who worked in Voit's laboratory with Rubner, was the first to bring the technique to this country. He started the first human calorimeter in 1892 at Wesleyan University, in Middletown, Connecticut, in collaboration with Rosa, the physicist. Francis G. Benedict, working with Atwater, added improvements to the calorimeter at Wesleyan, and then in 1908, as director of the Nutrition Laboratory of the Carnegie Institution of Washington and with the assistance of Thorne M. Carpenter, built the well-known calorimeters for human and animal study in Boston.

Graham Lusk, a pupil of Voit and a close friend of Rubner, also brought the Munich influence to this country. When Lusk became Professor of Physiology at Cornell University Medical College, funds were provided for the construction of a respiration calorimeter large enough to study the metabolism of babies and dogs, which was completed in 1912. The next year Lusk was able to extend the research to the clinic through the installation of the Russell Sage Institute of Pathology calorimeter in Bellevue Hospital under the medical direction of Eugene F. DuBois.

Important as the calorimeter was in the advance of the science of energy metabolism, Rubner considered it only as a valuable laboratory tool, useful in expanding the broad problems of energy exchange in living matter. In a paper published at the time of his retirement in 1924, Rubner states that he considered the concept of the law of surface area as his most important contribution. He lists the discoveries resulting therefrom as follows: the isodynamic law and the caloric basis of metabolism; the physical regulation of body temperature; the specific dynamic action of foods; that metabolism in youth is essentially a surface area phenomenon; that in changes in bodily condition, such as starvation, the surface area law does not apply.

General adoption of the surface area principle as the most satisfactory method of comparing metabolism in humans was stimulated by DuBois and co-workers starting about 1914. Carefully controlled experiments had established the agreement between calorimeter and respiratory metabolism determinations for experimental periods shorter than those used by Rubner and Atwater. Benedict had developed accurate respiratory apparatus which was gaining increasing application in the clinic. Rubner's early data on the comparison of 24-hour metabolism among various species gave obviously high values per square meter since the influence of food and activity was not excluded. Thus it became apparent that conditions of minimal stimuli must be fulfilled to obtain reproducible and comparable results with human subjects. These conditions became the criteria for what was designated as "basal," "standard," or "postabsorptive" metabolism.

In the next two decades an extensive accumulation of basal metabolism data provided the figures for the modification of Meeh's surface area formula to include height as well as weight (DuBois). The Harris-Benedict prediction tables for normal basal metabolism also included age and sex. Respiratory metabolism studies spread widely and rapidly both in clinical research on abnormal metabolism, and in the extension of Rubner's discoveries of the influence of environmental temperature, food, growth, age, and nutritional condition of the body on normal metabolism. Calories per square meter per hour became the generally accepted method of expressing human metabolism, although its general application to animal metabolism data encountered many difficulties, particularly in an accurate comparison among different species. Thus a causal relationship between surface area and basal metabolism became a matter of considerable controversy among the authorities in human and animal physiology.

Rubner continued his wide range of interest in the subject, collecting information on the surface area-metabolism relationship in birds, aquatic animals, amphibians, and reptiles and on the chemical analysis and heat of combustion of their

dried tissues. Shortly before his death he vigorously defended the surface area law before the Prussian Academy of Science in which he was honored as co-secretary with Planck. It should be remembered that Rubner was dealing with fundamental metabolism and surface area in broad terms. No one questioned the evidence that in comparing a range of animals from the largest to the smallest the metabolic rate corresponded in general to the anatomical surface of the body rather than to body weight. But to the nutritionist dealing with agricultural animals, it is practical and accurate to measure body weight whereas surface area measurements are difficult and inexact. The available data have been analyzed by both Brody and Kleiber. Respectively, they recommend that body weight to the 0.7 power or to the 0.75 power be used as the reference unit for "metabolic body size" or "physiologically effective body size." The accuracy is sufficient for comparison between species of animals but is questionable for intraspecific use.

Since the passing of Rubner and Lusk the metabolic concept of surface area has progressed away from the anatomical toward the physiological interpretation. Extended research on heat loss and skin temperatures under various conditions has shown that several overlapping body surfaces are concerned in removing heat from the body. For example, the effective radiation (Bohnenkamp) area is smaller by 20 to 35% than the total surface area, depending upon body posture. Likewise the convection and evaporative areas are variable and difficult to define. Recent research on thermal stress has served to indicate the complex integrated reactions of the peripheral and central nervous systems and of the endocrines in maintaining thermal homeostasis with a minimum of deviation from a balance between heat loss and heat production. It seems quite possible that the growing interest in thermoregulation and heat loss may uncover the interplay of fundamental physiological mechanisms which will vindicate Rubner's faith in a broad causal relationship between surface area and basal metabolism.

The other discovery to which Rubner's name is most frequently attached is that of the "specific dynamic action" of foods, the increase in heat production which follows the ingestion of fat, carbohydrate, or protein, and is quantitatively different for each of these foodstuffs. In his early experiments Rubner saw that protein was much more potent than fat or carbohydrate in increasing the heat production of the body. He then tried the effect of meat at different environmental temperatures and found that the extra energy of specific dynamic action was lost from the body as extra heat when the room temperature was 30°C., but was used to keep the body warm when the room temperature was lowered to 4°C. On the contrary the extra heat from ingested meat could not be used for muscular work. The following experiments in 1910 demonstrated this fact. A fasting man performing 100,000 kg-meters of work increased his heat production 45% over the resting level. Without muscular work a protein diet raised the metabolism 27%. The protein diet plus the same work gave a 70% increase in heat production, thus showing the additive effect of specific dynamic action and muscular work. Rubner postulated that two specific forms of energy are released in the metabolism of protein, one which supplies energy directly for the maintenance of cell life and the other which is the free heat of intermediary thermo-chemical reactions. He never changed his belief that specific dynamic action is due to the heat production of intermediary metabolism. During Lusk's last visit with him in 1930 he refused to elaborate the concept but replied, "There are various possibilities."

Through Rubner's influence Lusk initiated his studies of specific dynamic action and published the results in the second paper (1912) of the Animal Calorimetry series which he and his colleagues continued through two decades. Many other investigators in this country and abroad have contributed to the search for the cause of these energy transformations following the ingestion of food. The early work was concerned with the varying amount of heat from the different individual

amino acids. Conflicting results were obtained, particularly with glutamic and aspartic acids, depending on the type of experiment and the nutritional state of the experimental animal. Extra heat production was compared with the nitrogen content of the amino acids, their glucogenic or ketogenic properties, their structural relation to hormones, and whether or not they were essential for growth, maintenance, or the formation of important cell constituents. Unexplained exceptions confronted each new theory. Lusk summarized the situation with the following statement: "The hypotheses which have been presented on specific dynamic action transcend one's power to coordinate them."

The development of the *in vitro* techniques for respiration studies of isolated tissues, cells, and enzyme preparations and more recently the use of isotope-labeled radicals in the intact animal as well as *in vitro* have opened the way to a broader understanding of energy transformations in intermediary metabolism. The tricarboxylic acid cycle, originally applied to carbohydrate oxidation, now appears to link carbohydrate, fat, and amino acids through a common two-carbon molecule, "active acetic acid." A new concept of the specific dynamic action of amino acids suggests that the metabolic breakdown of the amino acids in preparation for oxidation via the tricarboxylic acid cycle produces the waste heat. Transamination is proposed as the mechanism responsible for the variations in results on glutamic acid. Evidence is accumulating in favor of the energy-rich phosphate bond as a common intermediate essential link in energy transformations in living tissue. Despite these tremendous advances Rubner's dictum of "various possibilities" is still applicable.

Rubner visited this country in 1912 to attend the Fifteenth International Congress of Hygiene and Demography in Washington where he was honored as the international president of the Congress. One session was a notable symposium on specific dynamic action. The distinguished speakers were Rubner, Zuntz, Benedict, and Lusk. The following month (October 5, 1912) he delivered the Harvey Lecture in New

York on "Modern Steam Sterilization," thereby becoming an honorary member of the Society. He was elected a foreign associate of the National Academy of Sciences in 1924.

Rubner's friends have described him as a well-built man of striking presence, whose character was upright like his stature, searching for the truth with remarkable objectivity, a creative artist in research, a man of great proportions. A glimpse of Rubner's broad vision is found in his own words, "Mute and still, by night and by day, labor goes on in the workshops of life. Here an animal grows, there a plant, and the wonder of it all is not the less in the smallest being than in the largest."

WILLIAM H. CHAMBERS

LIVER STORAGE OF VITAMIN A BY MALE AND FEMALE RATS

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FOUR FIGURES

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In the most-used method for the bioassay of vitamin A the growth of rats on graded doses is taken as the criterion of potency. In another method the amount of vitamin A in the livers of dosed rats is taken as the criterion. Some workers who use the latter method grade doses in terms of body weight. Cross ('45) showed that grading is unnecessary for growth tests. Grading by weight now seems unnecessary for liver tests, but response depends on sex. For instance, Kimble ('39) and Callison and Knowles ('45) showed that female rats had more vitamin A in their livers than males. Ender ('34) found more vitamin A in the livers of cows than in those of bulls. Brenner, Brookes and Roberts ('42) found more vitamin A in the livers of female rats than in those of males after feeding liver oils. Esh and Sutton ('48) and Booth ('50) confirmed this with fish oils and carotene, but Lemley, Brown, Bird and Emmett ('47) observed no sex difference. Goodwin ('50) says that carotenoids are more abundant in the organs of females than in those of males in various species.

The difference in liver storage between the sexes may be caused by different growth rates or body weights, or may be connected more fundamentally with sexual dimorphism. This paper describes experiments designed to illuminate these hypotheses.

[1] Member of the scientific staff of the Agricultural Research Council.

METHODS

Dosing

Inbred piebald rats shortly after weaning at 21 days of age were caged individually and given a vitamin A-free diet of extracted casein [2] 17, sucrose 53, arachis oil 13, dried yeast 13, and salt mixture 4%. Each rat received 2 mg racemic α-tocopheryl acetate and 120 I.U. vitamin D_2 weekly. When growth ceased, dosing was begun. Carotene in arachis oil containing quinol, and halibut liver oil whether or not diluted, were given orally daily from a micrometer screw syringe. Portions of carrot or green leaf for a day's dose for a group of rats were weighed quickly, divided onto palettes, equalized by inspection, and given to individual animals. Portions were weighed concurrently for chemical assay of carotene.

Determination of vitamin A

Rats were killed with coal gas usually two days after the final dose. The livers and kidneys were removed, digested with 10% KOH and analysed (livers individually, kidneys usually pooled) for vitamin A by the method of Davies ('33) with such changes as extracting twice with ether. (See also Davies and Moore, '39.) Vitamin A (axerophthol) in the extract was determined with $SbCl_3$ photoelectrically.

Determination of carotene

Total carotene was assayed according to the technique of the Analytical Methods Committee ('50) and of Booth ('49): cold extraction of pigments by mixed light petroleum, acetone and quinol was followed by separation on an alumina-Na_2SO_4 mixture. To assess alimentary disappearance, carotene was estimated in the dose and in the feces collected from 5 to 70 hours after dosing. The "blank" pigment, previously excreted on the carotene-free diet, was subtracted from the fecal pigment (Booth, '47).

[2] Glaxo A/E or C/E.

Precision

Individual liver storage by rats of the same sex in the same group given the same dose varied widely. In general,

$$s = l^{0.74}$$

where l (liver) is the mean number of International Units of vitamin A found per liver for a group and s is the standard deviation of a single determination. Thus at low levels the scatter was high: the coefficient of variation rose rapidly for low mean storage values. Doses were therefore designed to give responses of over 50 I.U. per liver. The coefficients of variation for the two sexes were similar.

EXPERIMENTAL RESULTS

Liver deposit of vitamin A greater in females

After rats were given 4.45 mg of carotene in leaves during 17 days, the males had 290 and the females 4[illegible] I.U. vitamin A per liver. Other groups given 5.2 mg in carrots stored 315 and 470 I.U. In over 20 comparisons with carotene in oil and vegetables under various dosing conditions, more vitamin A was found in the livers of female rats than of males when both ingested the same quantity of carotene.

Intestinal absorption or digestion

Carotene is incompletely absorbed. If females absorb more than males, this might explain their greater storage. Judged by the disappearance of carotene during its passage through the alimentary tract, summarized in table 1, there was no sex difference.

Preformed vitamin A

Carotene is converted to axerophthol in the intestinal wall. To see if the sex difference in response was associated with efficiency of conversion, the process was bypassed by giving axerophthol in halibut liver oil. Liver analyses showed that females stored more than males even when given the preformed vitamin.

The effect of time on liver stores

Popper and Brenner ('42) gave fish oils to rats and observed that the liver vitamin A was lost by males sooner than by females. Bult and Sorgdrager ('38) had made a similar observation. That this sex difference held when carotene was used as source of the vitamin was shown by the following experiments.

A batch of rats, each having consumed the same amount of carrot, was divided into groups which were killed at intervals up to 21 days after the last dose. Liver stores in

TABLE 1

"Absorption" of carotene during alimentation

SOURCE OF CAROTENE	RATS PER GROUP		CAROTENE INGESTED	CAROTENE IN FECES			DISAPPEARANCE	
	m	f		m	f		m	f
			μg	μg per rat			%	%
Green leaves	6	5	108	37.5	37.5		65	65
Green leaves	2	3	120	62	60		48	50
Oil	6	7	178	45	48		75	73
Oil	4	5	175	47	51.5		73	71
Carrot, cooked	5	5		various			45	42
Carrot juice	3	8		various			62	60
						Mean	61	60

males were lower than in females and both diminished at about the same rate: the liver-time curves for males and females were parallel, not divergent. When green leaves were fed as the source of carotene, there were similar sex differences but the diminution with time was slower in each sex.

Were the different levels in male and female livers due to immediate storage of different proportions of the dose, or to its being used up faster by males during the dosing period? If the amount stored immediately after one dose could be determined, then differential use during the interval between dosing and liver assay would be eliminated; i.e. the available surplus at zero time should be the same in both sexes. To

secure reliable response, the total dose must be large and spread over several days. Moreover, as shown below, single-dose response is not immediate. Hence the vitamin A deposited at once cannot be measured directly but has been estimated as follows. Experiments were grouped according to length of dosing period and the means plotted in figure 1. Extrapolation to zero dosing time gave a liver ratio value of 1.12. Thus males deposited less than females even from the same initial surplus.

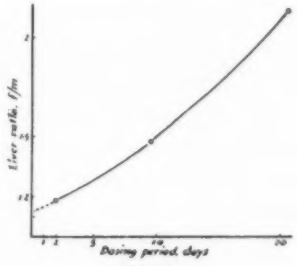

Figure 1

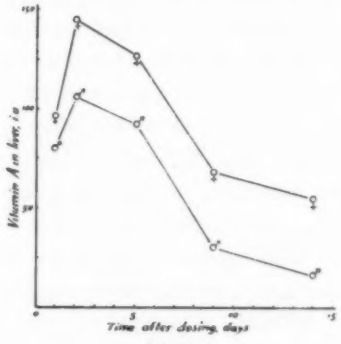

Figure 2

Fig. 1 The effect of dosing period on the female-to-male liver storage ratio of vitamin A. Each point represents the average of 8 or 9 experiments with a total of over 500 rats.

Fig. 2 Average liver stores of male and female rats, each given 500 I.U. of vitamin A at zero days.

With halibut liver oil a large amount of vitamin A can be given in one day, thereby partially obviating the complication of metabolism during the ingestion period. At 46 days of age 36 rats were each given 500 I.U. of vitamin A. Groups were killed at intervals. The results given in figure 2, show that maximum storage was reached only after two days and that the sex difference was observed initially. Another 36 rats from the same batch, each given 1,250 I.U. during two days, accumulated maximum storage only after 5 days. Another 70 rats were each given 640 I.U., with similar results.

It is concluded that liver stores are less in males than in females from the start, that both lose their stores at about the same rate (International Units per rat per day), and hence that the ratio (f/m) increases with time. This explains why the ratio increases with length of dosing period.

Relation with growth

Since vitamin A is essential for growth, faster-growing rats might use more of the vitamin, and therefore have less surplus for storage than slower-growing rats. Growth rate and storage might then be inversely related. Males grow faster than females: the mean of the ratios — male growth during the dosing period divided by female growth — in 24 groups comprising 217 rats in this study was 1.72, and the coefficient of variation of the 24 ratios was only 5.6. High and Day ('51) concluded that the sex liver difference was due chiefly to differences in growth. Correlation between growth and storage was therefore sought as follows.

Within a group of rats of one sex receiving the same dose, growth rates varied from rat to rat. The root mean square of the coefficients of variation for the rates in 26 groups of males and 27 groups of females was 18.3. The individual liver storage of vitamin A also varied. The 258 rats were used to calculate the correlation between growth rate and storage level within these 53 groups. This was — 0.02 for males, — 0.08 for females and — 0.05 for all rats. In other words, about the same percentage of a given dose was stored by faster-growing rats as by slower-growing rats of the same sex. These correlations, which are not significant ($P = 0.05$), make it improbable that greater growth rate was the cause of males' storing less surplus vitamin A.

As is shown below (table 2), older rats stored less of a given intake than younger rats which were growing faster. This observation also confutes the growth hypothesis.

Relation with body weight

More surplus might be stored by small animals than by larger animals from a given intake. Male rats are normally bigger than females and thus, if weight affects storage, would store less. The finding by Cross ('45), of no correlation between weight and growth on dosing A-free rats with carotene, is against that possibility. The body weights of 166 males and 191 females in certain of the present experiments, midway through the dosing period, were recorded. Within any isogestic group of the same age and sex the individual weights varied from rat to rat. The vitamin A found per liver also varied. The correlation for males was

TABLE 2

Effect of age or body weight on liver storage of vitamin A from carotene ingested

CAROTENE IN	AGE [1]	WEIGHT [1,2]	DOSE	LIVER A [2]
	days	*gm*	*mg*	*I.U.*
Oil	62	120	1.74	224
	91	144	1.54	105
Leaves	62	116	1.42	200
	108	154	4.00	220

[1] Half-way through the dosing period of 22 days.
[2] Mean for males and females.

-0.17 ($P = 0.03$), for females 0.02, and for both sexes -0.09 ($P = 0.11$). This hint that large males stored less than small males is not decisive enough to account for the different storage by males and females.

Seemingly the problem of whether body size controls storage could be solved by comparing rats of different ages and therefore different weights. However, the question arises, how much vitamin A must rats have for health between depletion and the beginning of the delayed test? The difficulty was suppressed rather than solved by using large doses. Young rats of both sexes were used at depletion to compare with older rats which had received barely enough of the vitamin for maintenance. Table 2 shows that twice

as much carotene was needed by larger as by smaller rats for similar liver storage. However, these results do not prove that body size is the dominating factor, because the larger rats were older than the smaller ones. It was not clear whether age or weight determined the storage level. There is evidence from other experiments that the absorption of vitamin A is impaired by vitamin A deficiency. The older rats had been deficient a long time.

Storage by very young rats

The difference in size between young males and females is negligible. Weanling rats were divided into groups and given a large dose each of carrot exceptionally rich in carotene (590 p.p.m.). Other weanlings were given halibut oil. The males weighed 38 and the females 37 gm. The results of 5 comparisons showed that males stored about the same as females. This supports the body weight hypothesis. But it is arguable that such immature rats do not yet show every sex dimorphism.

As rats grow the male/female weight ratio increases. Results of 23 experiments showed no correlation between body weight ratios and liver storage ratios for the sexes. Were weight the determining factor in liver storage, a positive correlation would be expected.

The effect of age

An attempt was made to separate the effects of age and weight as follows. Female rats were given a vitamin A-free diet supplemented with leaves and carrots supplying 32 μg of carotene per rat weekly — enough vitamin A for maintenance but not for liver storage. At 37 weeks of age the rats had almost finished growing. Ten were given two doses of halibut oil (1,240 I.U., total) and after 14 days their livers and kidneys contained 414 ± 22.3 [3] and 5 I.U. vitamin A, respectively. Their sisters were kept on the diet until they were

[3] Standard error of the mean.

39 weeks older but only 20 gm heavier. There were occasional signs of deficiency and three rats died. The 11 survivors were given two doses of halibut oil (1,575 I.U.) and 14 days later their livers and kidneys were found to contain 519 $\pm$ 23.6 and 13 I.U. Thus the older rats stored vitamin A as efficiently as the younger ones, 34% being recovered in each group. In another experiment, differing as to times, doses, and so forth, the same conclusion was reached.

If efficiency of storage is unaffected by age, the results in table 2 are best explained by the inverse weight hypothesis.

TABLE 3

The effect of litter size on liver storage and weight of rats
(Each rat was given 510 I.U. vitamin A in halibut liver oil)

Rats per litter	Two		Twelve	
Sex	m	f	m	f
Rats per group	3	6	9	8
Weight per rat, gm [1]	138	115	78	74
S.e., gm		5.0	4.4	1.5
Growth rate [2]	48	43	38	34
I.U. per liver	78	99	73	95
S.e., I.U. per liver		16.7	11.7	11.3

[1] Before killing at average age of 43 days.
[2] Mean increase in grams in 13 days prior to sacrifice.

The effect of underdevelopment on liver storage

Weanling body weights vary with litter size because the mother's milk must be shared. In each of 6 litters all the young except one male and one female were killed at birth. Two other litters were not so reduced and comprised 6 males and 6 females each. All were weaned as usual to the vitamin A-free diet. At 40 days of age, when twins weighed nearly twice as much as duodecets, rats from each litter were given 510 I.U. of vitamin A in halibut oil in two days. On the third day the rats were weighed, killed, and the livers analysed. The large females in twins stored more vitamin A than the *smaller* males in duodecets. Table 3 shows that the weights

of the rats, as controlled by litter size, had no effect on the storage by males or by females. The weight difference between the sexes was lower for duodecets than for twins, as might be expected from the observation of Jones ('51), but the liver ratios were the same for each litter type. Results with litters of 8 — e.g., males weighing 103 gm stored 77 I.U. per liver — fitted into the scheme but are omitted from the table. The difference between litters cannot have been caused by greater liver reserves in twins, for there was negligible vitamin A in the livers of 15 undosed rats; and in another experiment female twins given the A-free diet had, at 43 days of age, only 9 I.U. per liver against 2 I.U. in duodecet male livers. The female twins were growing faster than the male duodecets. Hence the low storage of the duodecet males cannot have been due to fast growth.

Growth on a limited regular intake of vitamin A

During bioassay by growth the dose of vitamin A given to rats is designedly too small for maximum growth. On this small dose, males grow faster than females. In 12 widely separated tests on different materials the average growth ratio was 1.43 (s.d. = $\pm$ 0.182). The faster growth of males suggests that their need of the vitamin for growth is no greater than that of females.

Growth and survival on limited single doses

Mayer and Krehl's ('48) male rats showed deficiency symptoms sooner than females. But when rats in this laboratory were given the vitamin A-free diet prior to dosing in 8 bioassays in which cessation of increasing body weight was the criterion of vitamin A deficiency, females "ran out" only about one day later than males. This was surprising because (fig. 2), after ingesting similar amounts of vitamin A, males lost their liver stores sooner than females. Perhaps young males have, at weaning, larger reserves than females. The results of liver tests on 7 groups of about 13 rats each,

averaging 38 days of age, on the vitamin A-free diet refute this explanation: males had 9.1 and females 7.6 I.U. — a difference of only 1.5.

Storage in kidney

That male rats lose their liver stores of vitamin A sooner than females, yet survive equally, suggests a reserve elsewhere which is greater in males than in females. Most of the stores are usually assumed to be in the liver, with small amounts in the kidney. Johnson and Baumann ('47) and Moore and Eden ('51) found more vitamin A in kidneys than in livers of rats when the total was small. Johnson and Baumann ('48) found that kidney stores rose as liver stores fell in rats on a deficient diet. Sex differentiation in kidney storage was therefore studied. Meanwhile Moore and Sharman ('50) reported more vitamin A in male than in female kidneys after dosing rats for a month. This was confirmed using single doses (Booth, '50) and has been amplified as follows.

Male rats on the A-free diet at 33 days of age had 5.5 and females had 4.7 I.U. per kidney pair. At 41 days of age males had 1.3 and females 2.0 I.U. Thus sex difference on this diet was negligible.

Similar rats were each given 500 or 1,250 I.U. of vitamin A in halibut oil and killed two days later. The kidneys of males and of females contained 10 I.U. of vitamin A per pair. In contrast to liver, the amount of vitamin A stored in the kidneys was scarcely affected by increasing the dose two and one-half times. Other experiments confirmed both conclusions, but when assayed more than two days after dosing more vitamin A was found in male than in female kidneys. This is seen in figure 3, drawn from the pooled results of several experiments in which 238 vitamin A-free rats were given 480 to 1,250 I.U. of vitamin A in halibut oil, divided into groups and sacrificed at intervals. Male kidney levels rose with time, as stated by Johnson and Baumann ('48) for "rats" without mention of sex, but in females the rise was less marked.

Kidneys were usually pooled, but in one experiment they were analysed in pairs. The standard deviation was independent of the level of response and had a pooled value of 3.53 I.U. for the 5 groups of 7 males and 5 groups of 7 females. Hence the standard error was 1.34, which indicates the precision of figure 3.

The sex difference in kidney storage cannot account for differential liver storage because the kidney difference is too

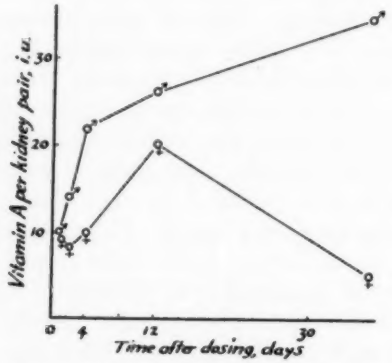

Fig. 3 Effect of time on kidney stores of vitamin A in rats given halibut liver oil on zero day.

small and the liver difference anticipates the kidney difference. Both differences may be seen by comparing figures 2 and 3.

Responses by males and females to graded doses

Rats were given graded doses of halibut oil during 9 days and killed two days later. Several conclusions are drawn from the results of 64 liver analyses summarized in figure 4. (1) Rats given lower doses, though not represented in the figure because their liver responses were zero, nevertheless grew satisfactorily. (2) The dose had a threshold value below which no vitamin was found in livers. This value depends on length of dosing period. (3) The threshold value was higher

for males than for females. In this experiment the extrapolated values were 340 and 270 I.U. (4) The response for each sex was almost linear over a 60-fold dose range. (5) The extrapolated male and female curves met at zero dose value but below zero liver response. (6) The difference between male and female storage was not constant: the response curves had different slopes. The difference was proportional to the dose,

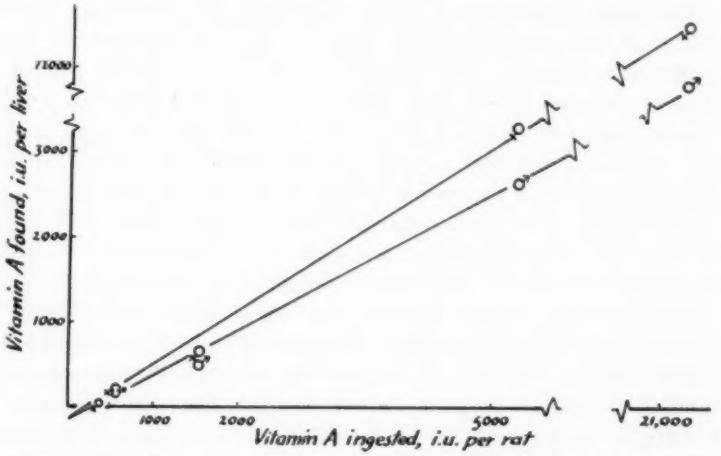

Fig. 4 Dose-response curves for rats given halibut oil over 9 days. The scales have been divided by three for the higher points.

of which under these conditions (9-day dosing period) it was 11%. In other words, 11% more of a given dose disappeared in the male than in the female.

It follows from items 3 and 4 that

$$l = k(i - t),$$

where l is the vitamin A found in the liver, i is that ingested and t is the threshold. In these experiments $k = 0.53$ for males and 0.63 for females.

More was ingested by males than by females for a given storage level. The percentage difference varied a little with level but near the middle of figure 4 it was 24%. Males weighed

23% more than females. The correspondence of the two differerences supports the body weight hypothesis. If faster growth caused greater disappearance in males, a constant difference would be expected in the liver responses, independent of dose, whereas a proportionate difference was found.

Castration

Bult and Sorgdrager's ('40) castrated rats lost their liver stores of vitamin A at rates intermediate between those for males and females. The present author has studied castration as follows. Male rats were castrated and females were ovariectomized when 32 days old. At 46 days of age they, and normal litter mates, were given halibut oil. After 2, 26 and 46 days rats from each group were killed. Castrated males had slightly more liver vitamin than normal males and lost their stores very slightly more slowly; i.e., castration tended to induce femininity. The ovariectomized females stored more vitamin A than normal females, lost their stores very slightly more slowly and grew faster. In growth rate they resembled males, but in vitamin A storage the small difference from normal females was away from the direction of the males. Castrated males on each date had kidney vitamin A values between those of males and females. Ovariectomized females also stood between males and females, closer to females.

CONCLUSIONS

Items 5, 8, 9 and 11 in the summary disprove the growth rate hypothesis for differential liver storage between the sexes. Moreover, different growth rates could hardly account for the difference found immediately after dosing.

Another hypothesis, that liver storage is inversely related to body weight and males therefore store less vitamin A in their livers because they are larger than females, is supported by summary items 7 and 11. Items 6 and 8 and the lack of correlation between weight ratios and liver ratios, however, provide evidence against the hypothesis. The logic

of these deductions is explained in the relevant sections under Experimental Results.

The observations that (1) sub-threshold doses induced no liver storage, (2) rats given sub-threshold doses grew normally, (3) sex difference in storage was proportional to dose, and (4) only half the vitamin ingested appeared in the liver, suggest that vitamin A is also stored elsewhere than in the liver, and in greater amount in males than in females. The alternative site cannot be the kidney, for the amount there was small and did not rise with increased dose. No other tissue is reputed to contain appreciable amounts. Possibly the vitamin which disappears is destroyed: this hypothesis, however, fails to explain how rats survive after their liver stores are depleted. Evidence accumulates for the existence of a form of vitamin A not detected by recognized chemical tests (Kaunitz and Slanetz, '50; Le Gallic, '47). Readier storage of this vitamin by males than by females would explain the survival anomaly.

Thus it is not known what happens to the unrecovered proportion of the ingested vitamin. Nor is the problem of unequal disappearance in the sexes solved, but it is hoped that light has been thrown on it. Preliminary communications show that several workers are studying the effects of hormones on vitamin A metabolism. Their results may clarify the problem. Another approach might be to use species in which females are as large as males. It would be interesting too to know whether other axerophthoids are stored unequally by the sexes.

Whatever is the explanation of differential storage, two practical conclusions can be stated; namely, that in bioassaying by the liver storage method, (1) doses should not be graded according to individual body weights, although males may be given larger doses than females, and (2) data for males and females must receive separate arithmetical treatment.

SUMMARY

1. Vitamin A-free rats were given carotene in oil or vegetables, or halibut liver oil, then killed. Vitamin A was assayed by the $SbCl_3$ test in the livers or kidneys or both.

2. The standard deviation of a liver determination of vitamin A within any one group varied with the 0.74th power of the mean liver value for the group.

3. After they had ingested carotene, more vitamin A was found in the livers of females than of males. Both lost their stores at similar rates. Hence male livers depleted sooner than female and the female/male storage ratio increased with time.

4. The absorption efficiency of carotene was not linked to sex.

5. Neither sex showed correlation between storage and growth rate.

6. Correlation was negligible between storage and body weight among rats of one sex at the same age similarly dosed.

7. Infant rats had negligible sex differences in body weight and liver storage. Young rats stored more than middle-aged rats from the same intake, but old rats stored the same proportion as middle-aged rats of similar size.

8. Twin females, though weighing more and growing faster than duodecet males, stored more vitamin A.

9. On vitamin intakes too low for maximum growth, males outgrew females. After being given a vitamin A-free diet from weaning, or after one dose of vitamin A, males showed deficiency symptoms only slightly sooner than females.

10. Males and females had similar kidney levels shortly after a dose. The level in male kidneys increased with time, but in female kidneys no comparable rise was found.

11. Male and female dose-response curves had different slopes. The difference between male and female liver stores at different doses was a constant proportion (11%) of the dose. The threshold dose, below which there was no storage, was higher for males than for females.

12. Items 5, 8, 9 and 11 show that the sex-liver-storage difference cannot be related to different growth rates. Another hypothesis, that liver storage is inversely controlled by body weight, is supported by items 7 and 11, refuted by 6 and 8. This and other possibilities are discussed under Conclusions.

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NUTRITIONAL STUDIES OF THE CHINCHILLA, WITH SPECIAL REFERENCE TO ASCORBIC ACID AND THIAMINE

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INTRODUCTION

No studies of the nutrition of the chinchilla, *Chinchilla brevicaudata* or *Chinchilla laniger*, are available in the literature. The basic procedures used here were adapted from those employed previously by Claussen and Clark ('43), Cooperman et al. ('43), Hamilton and Hogan ('42), and Hogan and Hamilton ('44) in studying the vitamin requirements of other rodents.

The present status of the chinchilla industry places two rather severe restrictions on the use of these animals for experimental purposes. First, the cost of the animals themselves (approximately $1,000 per breeding pair) limits the number available. Second, the necessity on the part of commercial breeders to keep their females for breeding stock almost eliminates the use of females in experiments where mortality is likely to be high.

Because of the fact that the experiments reported here employed limited numbers of animals, the conclusions drawn are considered tentative, pending further work with larger groups of animals and perhaps several changes in the purified diet.

EXPERIMENTAL

Management of animals

The chinchilla is a strictly herbivorous rodent native to South America. Bickel ('31) and Prell ('34) have made ana-

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tomical studies of the chinchilla. Pierce ('44) and the U. S. Fish and Wildlife Service ('44) have described standard practices in herd management.

In the present investigations the animals were housed in wire cages ($\frac{1}{2}$" mesh) 18" × 18" × 18", to the outside of which was connected by means of an open door, 8" × 9", a sheet metal "nest box," 11" × 11" × 8". The nest box also had a wire bottom. Each animal was separated from that below it by a sheet metal dropping board. Water was given ad libitum from inverted bottles. The temperature was maintained between 50° and 60°F. The animals were allowed to dust themselves in clean Fuller's earth for 5 to 10 minutes daily.

Procedure, Part I

In preliminary experiments using mature animals, a variety of purified diets were completely rejected. Starch, dextrins, and sucrose as carbohydrate sources, the addition of steam distillates from alfalfa and clover as flavoring agents, and the addition of monosodium glutamate as a flavor intensifier all failed to improve acceptance. Purified diets in the form of mashes, pellets, and moistened (dustless) powders were also rejected by mature animals.

On the basis of preliminary experiments with weanlings, a purified diet (ration 1, table 1) was prepared with the vitamin concentrations expressed in terms of milligrams or micrograms per 15 gm ration, since the animals eat an average of 15 gm of ration per day during the growth phase studied.

Ten animals received from commercial breeders the day of weaning (age, 5 to 11 weeks) were divided into three groups. In all experiments reported here the animals were fed ad libitum. Group 1, consisting of two animals fed a "ranch-type" ration of rabbit pellets and a fermentation product of alfalfa meal,[2] served as controls. Group 2 con-

[2] Enzymalfa, Jeffries Laboratories, Roanoke, Virginia; a product prepared by fermentation of alfalfa meal and sold as a supplementary feed for poultry. "Wayne" Rabbit Pellets.

sisted of 4 animals fed ration 1. Group 3 consisted of 4 animals fed ration 1 plus 2 mg ascorbic acid in a sucrose solution fed orally by pipette each day. The selection of animals for groups 2 and 3 was made on the basis of their

TABLE 1

Composition of purified diets used

CONSTITUENTS	RATION	
	1	2
Sucrose[1] (%)	48	45
Casein (%)	25	25
Cellulose (%)	17	20
Vegetable fat (%)	5	5
Ashed alfalfa (Roine et al., '49; %)	2.5	2.5
Salt mixture (U.S.P. XII, no. 2; %)	2.5	2.5
Biotin (μg/15 gm)	2.0	4.0
Vitamin B_{12} (μg/15 gm)	0.5	1.0
Thiamine hydrochloride (mg/15 gm)	0.2	0.4
Riboflavin (mg/15 gm)	0.3	0.6
Pyridoxine hydrochloride (mg/15 gm)	0.2	0.4
Calcium pantothenate (mg/15 gm)	0.3	0.6
Niacin (mg/15 gm)	0.7	1.4
p-Aminobenzoic acid (mg/15 gm)	14.0	28.0
i-Inositol (mg/15 gm)	35.0	70.0
Folic acid (mg/15 gm)	0.07	0.14
Choline chloride (mg/15 gm)	15.0	30.0
Menadione (mg/15 gm)	0.5	1.0
Alpha-tocopherol acetate (mg/15 gm)	30.0	60.0
Vitamin A (I.U./15 gm)	50.0	100.0
Vitamin D (I.U./15 gm)	10.0	20.0
$MnSO_4 \cdot 4H_2O$ (mg/15 gm)	3.05	3.05
$ZnSO_4 \cdot 7H_2O$ (mg/15 gm)	3.05	3.05
$CuSO_4 \cdot 5H_2O$ (mg/15 gm)	0.6	0.6
$CoCl_2$ (mg/15 gm)	0.5	0.5
KI (mg/15 gm)	0.6	0.6

[1] Sucrose supplied as Domino cane sugar; casein as "vitamin-test," General Biochemicals, Inc.; cellulose as "non-nutritive diet," General Biochemicals, Inc.; vegetable fat as Crisco; and vitamin B_{12} as experimental animal protein factor, Merck and Co., Inc.

willingness to accept the sucrose solution voluntarily because it was found that force-feeding made the animals extremely nervous. The animals were weighed weekly for 15 weeks.

TABLE 2

Growth rates of individual chinchillas with and without vitamin C

(grams per week)

ANIMAL NO.	GROUP 1		GROUP 2				GROUP 3			
	1	2	3	4	5	6	7	8	9	10
Initial weight (gm)	175	190	135	280	230	175	205	225	130	160
Weeks 1–7	(Ranch diet)		(Ration 1)				(Ration 1 plus 2 mg ascorbic acid per day)[1]			
	14	14	14	—2	—3	6	11	16	Died	11
Weeks 8–11	(Ranch diet)		(Ration 1 plus 2 mg ascorbic acid per day)[2]				(Ration 1 plus 2 mg ascorbic acid per day)[2]			
	4	7	5	—1	7	—1	18	10	..	9
Weeks 12–15	(Ranch diet)		(Ration 1, no ascorbic acid, other vitamins doubled)				(Ration 1, no ascorbic acid, other vitamins doubled)			
	16	9	8	9	15	8	18	12	..	9
Average gain/week (gm)	10.6		5.8				12.5			

[1] In sucrose solution given orally by pipette.

[2] Mixed into the dry ration each day just prior to feeding.

During the 8th through 11th weeks the procedure was altered in that 2 mg ascorbic acid/15 gm ration were mixed into the feed of all animals on purified diets (but not that of the controls) each day just prior to feeding.

During the 12th through 15th weeks all animals in groups 2 and 3 were offered the same purified diet except that the concentrations of all the vitamins with the exception of ascorbic acid were doubled. No ascorbic acid was fed to either group 2 or group 3. The results in terms of growth rates are shown in table 2.

Procedure, Part II

Ten animals received from commercial breeders the day of weaning (age 5 to 8 weeks) were divided into 4 groups. All animals were offered ration 2, table 1, without thiamine for one week. From the second week on, group 1 (two animals) continued throughout the experiment on the thiamine-free diet. Group 2 (three animals) was fed the same purified diet with 0.05 mg thiamine/15 gm ration. Group 3 (three animals) was fed the same diet with 0.10 mg thiamine/15 gm ration. Group 4 (two animals) was fed the same diet with 0.40 mg thiamine/15 gm ration. All animals were weighed weekly for 7 weeks. The results are shown in table 3.

DISCUSSION OF RESULTS

Part I

No data on the normal growth of animals from the same stock as the experimental animals is available outside of the two used as controls in this experiment. It is therefore impossible to state whether the growth attained (10 to 12 gm/week) is typical of the healthy animal under ranching conditions. Data from other stock (National Chinchilla Breeders of America, '49) show an average growth rate over a comparable age span of 17 gm/week, but the number of animals is not given and the individual rates ranged from 8.7 to 24.8 gm/week. Because only small groups of animals were

used here, it can only be concluded that the growth of weanlings on the modified diet appears to have been nearly normal.

At the end of the 7th week the poor growth response of the animals in group 2 (without vitamin C) appeared to indicate that vitamin C is a dietary essential for the growing chinchilla. The possibility remained, however, that the selection of animals for groups 2 and 3 was biased. Should

TABLE 3

Weight responses of weanling chinchillas to various levels of thiamine

	GROUP 1, RATION 2 WITHOUT B_1		GROUP 2, RATION 2 WITH 0.05 MG B_1/15 GM			GROUP 3, RATION 2 WITH 0.1 MG B_1/15 GM			GROUP 4, RATION 2 WITH 0.4 MG B_1/15 GM	
Animal no.	1	2	3	4	5	6	7	8	9	10
Initial wt. (gm)	230	250	210	210	210	220	190	155	185	170
Net gain or loss in wt.					(gm)					
Weeks after depletion										
1	5	—15	0	5	—5	—5	—10	10	15	15
2	10	—5	5	0	5	—5	—10	30	40	20
3	15	—5	35	15	0	25	—10	45	25	30
4	25	0	35	15	0	30	10	45	55	60
5	25	—5	20	25	0	40	10	60	65	60
6	35	—10	50	25	0	50	15	55	65	70
Ave. net gain (gm)	12.5		25			40			67.5	
Ave. gain/week (gm)	2.1		4.1			6.7			11.3	

the poor growth of group 2 have been due to a deficiency of vitamin C, then the addition of the vitamin to the ration, as was done in the second phase of the experiment, would have been expected to result in improved growth. This was not found to be the case.

Since the difference in the growth rates of the two groups did not appear to be the result of a vitamin C deficiency, it was thought that perhaps one of the other vitamins was present in the ration at a slightly submarginal level. Under

such conditions, those animals possessing a cecum microflora that was active in the synthesis of the deficient vitamin might show a normal growth response, while those with a slightly less active flora might show impaired growth. The fact that one animal in group 2 grew as well as either the controls or the average of group 3 supports the conclusion that some factor other than vitamin C was causing the difference between groups 2 and 3. The possibility also existed that the chinchilla requires some unknown vitamin for growth not present in the purified diet, which was being supplied to some of the animals by bacterial synthesis and not to others.

In the third phase of the experiment, where vitamin C was omitted from the diets of both groups and the amounts of all other vitamins were doubled, the animals of group 2 showed a marked increase in growth rate, while those in group 3 continued to grow at about the same rate as before. This growth in group 2 was in contrast to the failure of the animals to show appreciable growth during the preceding 6 weeks. The apparent growth of animal 5 during the 8th to 11th weeks is attributed to the fact that he became constipated during the 6th week, stopped eating, and lost weight badly. Recovery came during the 8th week, without change in diet or handling, and is reflected in the increased weight shown between the 8th and 11th weeks.

This evidence indicates that vitamin C is not a dietary essential for the growing chinchilla.

Part II

Animals receiving no dietary thiamine showed a decided tendency to develop alopecia on the rear regions of the body, including the hind legs down to the toes. Animal 6 (group 3) first showed the alopecia during the depletion week, but began to grow new fur about one week after thiamine was included in the diet. Both animals in group 1 (no thiamine) began to show alopecia during the 4th week and failed to grow new fur. Beginning during the 4th week both animals in group 1 also showed signs of impaired co-

ordination when rolling in their dust pans. By the end of the 7th week neither animal was able to roll over completely without a great deal of struggling.

The probable error of the data relating to group 4 is 2.38, making that group significantly different from group 3 at a 1% level. The data indicate that dietary thiamine is essential for the growing chinchilla. The rate of growth attained by group 4 was of the same order of magnitude as that obtained in the preceding experiment by either the control group or group 3 (10 to 12 gm per week). Since the animals ate an average of 15 gm of ration per day, it would appear that the minimum daily requirement of the growing chinchilla for thiamine probably lies somewhere between 0.1 and 0.4 mg.

Increasing the cellulose to 20%, as in ration 2, table 1, resulted in more normal size and texture of the feces. Ration 2 is therefore considered an improvement over ration 1.

SUMMARY

1. The development of a purified diet for chinchilla weanlings which apparently will support normal or nearly normal growth for 15 weeks is reported. The diet is similar to those used for other rodents except that it contains 20% roughage, lower levels resulting in constipation.

2. Based on the use of such a diet, evidence is presented indicating that vitamin C is not a dietary essential for the growing chinchilla.

3. The minimum daily thiamine requirement of weanling chinchillas for growth appears to be between 0.1 and 0.4 mg.

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STUDIES ON ESSENTIAL FATTY ACID DEFICIENCY IN THREE STRAINS OF MICE [1]

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TWO FIGURES

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Although essential fatty acid deficiency has been studied in detail in the rat, very little work has been done with the mouse. White et al. ('43) described the syndrome produced with a fat-free diet in albino mice of their own laboratory strain. They found that the deficiency could be cured or prevented by the addition of lard to the diet. In the present paper we report our observations on the fatty acid deficiency syndrome in three highly inbred strains of mice. While this work was in progress, Decker et al. ('50) reported that they had been able to produce a chronic deficiency in mature mice.

EXPERIMENTAL

Mice [4] of the C57 black, dba, and C3H strains were used. The composition of the diets is given in table 1. Each mouse strain was set up in 4 groups (table 2). Group I was a negative control group maintained on the deficient diet, P-54.

[1] This investigation was supported, in part, by a grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

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[4] The C3H and dba mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The C57 black mice were bred in our own stock colony.

Group II consisted of positive controls receiving the complete diet, P-53. Group III was supplemented with varying levels of methyl linoleate after development of the syndrome. Group IV was supplemented with 5 mg of methyl linoleate per day at the time the negative controls were placed on diet P-54.

The mice were started on the experiment when they weighed between 12 and 16 gm. Those receiving diet P-54 were allowed to develop the deficiency symptoms. When they reached this stage, which was accompanied by a loss of weight, a

TABLE 1

The composition of the basal diets

CONSTITUENTS	FAT-FREE (P-54)	CONTROL (P-53)	VITAMIN SUPPLEMENT PER KG OF DIET	
	%	%		
Casein, vitamin-free [1]	25	25	Thiamine hydrochloride	5 mg
Sucrose	68	53	Riboflavin	5 mg
Salt mixture [2]	5	5	Pyridoxine hydrochloride	5 mg
Ruffex	2	5	Biotin	100 mg
			Calcium pantothenate	30 mg
Lard	..	5	Choline chloride	500 mg
Hydrogenated vegetable fat [3]	.	10	Vitamin A [4]	67,500 I.U.
			Vitamin D [5]	5,000 I.U.
			α-Tocopherol acetate	200 mg
			Folic acid [6]	5 mg

[1] Labco.

[2] Osborne and Mendel.

[3] Crisco.

[4] Given in the form of a concentrate (1,000,000 I.U./gm) supplied by the Nopco Chemical Co., Harrison, N. J.

[5] Given in the form of Drisdol (Winthrop-Stearns Inc., New York, N. Y.).

[6] Kindly supplied by the Lederle Laboratories Division, American Cyanamid Co., through the courtesy of Dr. T. H. Jukes.

TABLE 2

Number of mice on various dietary regimens

STRAIN	GROUP			
	I	II	III	IV
C57	8	18	22	9
dba	14	4	10	4
C3H	13	4	5	4

certain number of the animals were given 2, 5 or 10 mg methyl linoleate per day. In the case of the C57 and dba mice, the development of the neck lesion was used as the criterion in determining when to start the treatment. In the case of the C3H mice, a significant drop in weight was the determining factor. A cure was considered to have taken place when the external signs of the deficiency disappeared and the animals had gained weight.

The effect of pre-depletion of the weanling on the time required for the development of the syndrome was determined by depletion of the mother during the latter part of lactation.

The mice were weighed on alternate days and were given the indicated supplements daily. The alcohol solution of methyl linoleate was given by spreading the solution through the top layer of food in the feed cup. Water was supplied ad libitum.

RESULTS

Most of the symptoms noted with the three strains were the same as those found previously by White et al. ('43). These included a dermatitis of the skin and the extremities, scaliness of the ears, alopecia, and retardation of growth. A neck lesion, characterized by an initial alopecia of the neck followed by a dermatitis and lesioning, was also shown by all three strains. As is shown in table 3, in which data obtained on groups I and III have been included, most of the mice developed a "browning," which was a lightening in fur pigmentation in the lower dorsal region.[5] In two-thirds of the dba mice a "spectacle eye" condition was observed. This symptom was also noted occasionally in the C57 mice.

It was also found that pre-depletion of the C57 weanling resulted in development of the syndrome in less than one-half the time required with mice weaned on a stock diet containing all the nutrients. The effect of pre-depletion on the dba mice was even more marked; they developed the visible

[5] Preliminary studies by Miss Cecilia Torda in our laboratory indicate that a similar "browning" occurs in black rats (Evans-Long strain) kept on the deficient diet, P-54.

symptoms in less than one-third of the time required by mice weaned on a stock diet.

The daily requirement for methyl linoleate was found to be approximately 5 mg. As can be seen in table 4, 2 mg per day were totally inadequate for the cure of the syndrome.

TABLE 3

Average day of development of symptoms in fatty acid-deficient mice

SYMPTOMS	C57 (11)[1] (Stock)[2]	C57 (19)[1] (P-54)[2]	dba (15)[1] (Stock)[2]	dba (9)[1] (P-54)[2]	C3H (18)[1] (Stock)[2]
Dandruff	33 (11)[3]	10 (19)[3]	30 (2)[3]	9 (1)[3]	30 (14)[3]
Browning	47 (10)	22 (18)	50 (14)	..	50 (13)
Neck lesion	71 (9)	45 (18)	41 (10)	12 (6)	44 (11)
Alopecia	76 (8)	41 (14)	51 (9)	16 (6)	66 (9)
Ear thickening	103 (6)	40 (17)	43 (10)	11 (5)	66 (9)
Skin dermatitis	107 (6)	55 (9)	57 (7)	12 (8)	..
Tail dermatitis	113 (4)	56 (6)	56 (6)	40 (1)	55 (14)
"Spectacle eye"	..	..	46 (9)	11 (6)	..

[1] Figures in parentheses represent number of mice used.

[2] Diet on which the mice were weaned.

[3] Figures in parentheses represent number of mice which developed symptoms.

TABLE 4

Comparison of curative effects of varying amounts of methyl linoleate in the three strains

SUPPLEMENT PER DAY	C57		dba		C3H	
	No. of mice	No. of cures	No. of mice	No. of cures	No. of mice	No. of cures
mg						
2	11	0	3	0	..	..
5	9	8	5	5	5	4
10	2	2	2	2	..	..

Preliminary experiments indicated that a 3-mg level of methyl linoleate produced only a temporary, partial remission of the syndrome.

When the mice were given 5 mg of methyl linoleate per day before the development of the symptoms, they maintained normal growth at a slightly lower level than the positive controls and did not show any external symptoms of the deficiency.

The growth of these mice as compared with that of the positive controls is shown in figure 1 for C57 mice. Results with the dba and C3H mice were the same as those with C57 mice.

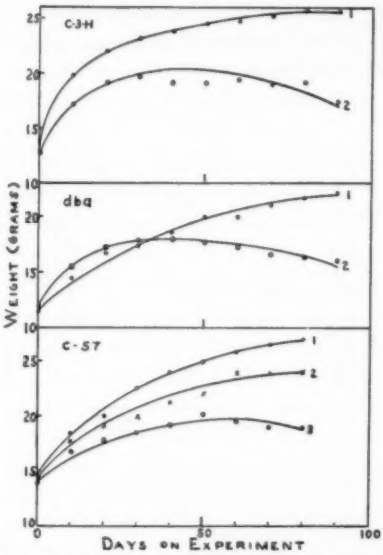

Fig. 1 Comparison of average growth rates for mice of the three strains receiving diet P-53 (curves marked 1), and for those receiving the deficient diet P-54 (curves marked 2 for the C3H and dba animals, and curve 3 for the C57 mice). The average growth rate for 4 mice of the C57 strain which were given 5 mg methyl linoleate per day prior to the development of the symptoms is also shown (curve 2).

In figure 1 are shown the curves of normal and deficient mice. Figure 2 shows the effect of supplementation with 5 mg of methyl linoleate per day on the growth of deficient mice.

In the case of 4 dba mice in which supplementation with 5 mg of methyl linoleate was discontinued after the cure of the deficiency, a loss in weight was noted, and three of these mice died before the syndrome appeared. The 4th mouse again showed the external symptoms of the deficiency before death.

Mice of the dba strain were found to be extremely sensitive to the essential fatty acid deficiency. As may be seen in table 3, this strain developed the syndrome more rapidly than either the C57 or C3H mice. In general, there was a high rate of mortality among the dba mice in the early stages of the experiment; about one-third of these mice died before the syndrome could develop.

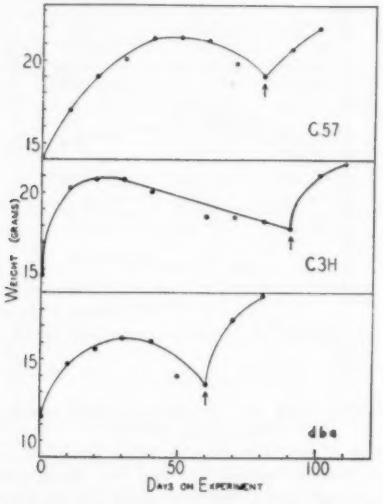

Fig. 2 Curves showing the effects of supplementation with 5 mg of methyl linoleate per day in deficient mice of the three strains. The arrows indicate the time when supplementation was begun.

Apparently there is some storage of the essential fatty acids in mice weaned on a stock diet, for it was found, in general, that the earlier the mice were started on the experiment after birth, the earlier did their symptoms develop.

Since mice which were supplemented with 5 mg of methyl linoleate per day, prior to the appearance of the external symptoms, developed normally, and since this quantity of the ester given per day was sufficient to cure the visible signs of the deficiency and to prevent recurrence of the syn-

drome, it appears that this level of methyl linoleate is the daily requirement of the mouse for this essential fatty acid.

SUMMARY

Essential fatty acid deficiency has been studied in mice of the C57 black, C3H, and dba strains. The most characteristic symptoms were: dermatitis of the skin and extremities, scaliness of the ears, alopecia, a neck lesion, and retardation of growth.

Pre-depletion of the weanlings, produced by placing the mother on a deficient diet during the lactation period, had a marked effect on the time of development of the symptoms.

The dba strain was found to be more sensitive to the deficiency than the C3H or C57 strains. Prophylactive and curative experiments indicate that under the experimental conditions used 5 mg of methyl linoleate per day satisfy the requirement of the mouse for linoleic acid.

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ASCORBIC ACID NUTRITURE IN THE HUMAN

I. TYROSINE METABOLISM AND BLOOD LEVELS OF ASCORBIC ACID DURING ASCORBIC ACID DEPLETION AND REPLETION[1]

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ONE FIGURE

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Data as to dietary intakes and blood serum levels of ascorbic acid of a large population group have been compiled during recent nutritional survey studies in the Northeastern region.[2] Such data yield information concerning the ascorbic acid nutriture of the group at the time of the study, but without more detailed investigations they do not yield information concerning the past ascorbic acid nutriture of the group. The ascorbic acid content of the white cells and platelets is thought to be a more adequate measure of the tissue content of the vitamin. Investigations of this relationship have been made by Crandon et al. ('40), Butler and Cushman ('40), Lowry, Bessey, Brock and Lopez ('46), and the Medical Research Council of England ('48). Lowry and co-workers, in their study, which involved 100 subjects maintained for 8 months on diets containing varied amounts of ascorbic acid, showed the presence of a direct relationship between ascorbic acid concentration in serum and white cells at the end of the

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[2] Bulletins on the *dietary findings* and *chemical findings* of the Nutritional Status Project will be published by the Northeast Region NE-4 group.

study period. Such a relationship might enable one to assess the level of past ascorbic acid nutriture from the more easily obtained serum values, unless the individual had a highly erratic intake of the vitamin. It was thought, however, that more information was needed concerning the relationship between serum and white cell values determined at frequent intervals while subjects were ingesting a diet deficient in ascorbic acid.

In addition, it was considered important to investigate the urinary excretion of "tyrosyl" compounds, because this excretion might give early indication of ascorbic acid depletion. Various workers (Sealock and Silberstein, '39, '40; Levine, Marples and Gordon, '39) have reported urinary excretion of abnormal amounts of tyrosine metabolites by guinea pigs and premature infants deficient in ascorbic acid. Rogers and Gardner ('49) have made similar observations with respect to scorbutic adult humans. To our knowledge, no study involving tyrosine metabolism during an induced suboptimum ascorbic acid intake in the adult human has been reported in detail.

This report is concerned with the urinary excretion of "tyrosyl" compounds and the ascorbic acid level of serum and white cells of 10 adult humans ingesting a diet containing 7 mg or less of vitamin C.

EXPERIMENTAL

Five men and 5 women volunteered to serve as subjects. They ranged between 21 and 32 years of age and on the basis of medical examination were judged to be in good physical condition.

The menus were planned on a three-day basis and were repeated throughout the experimental period of 82 days. A "core" diet consisting of eggs, meat (beef, pork, fowl), milk, stewed prunes, and canned pears was ingested by each subject. Cereal products and butter were added in various amounts to meet each subject's energy needs. Sugar, special hard candies, and soft drinks were allowed ad libitum but

in known amounts. With the exception of ascorbic acid, the diet was considered to be adequate in known dietary essentials for the human. The daily intake of ascorbic acid was 15 mg or less during the first two weeks; by treating the milk with heat, the ascorbic acid intake was reduced to 7 mg or less per day during the remainder of the depletion period. The diet contained variable amounts of pteroylglutamic acid, a compound which is known to inhibit hydroxyphenyluria when given in a high enough dose (Govan and Gordon, '49; Morris, Harper and Goldbloom, '50). It is doubtful that the amount present in an ordinary diet would have a retarding effect.

During the morning of the first day of the study, fasting samples of venous blood were withdrawn. After one week a balance period of 6 days was started. The subjects received 5 gm of L-tyrosine with each noon and evening meal during the balance period (a total of 10 gm per day). Complete urine and fecal collections were made, and at least one fasting sample of blood was withdrawn during each metabolic period.

Other balance studies, of at least 6 days each, were made after the subjects had been ingesting the diet for 31, 54 and 73 days. The last 4 days of the study (79 to 82 days) were devoted to saturation tests. During this time each subject received 800 mg of *l*-ascorbic acid (in 4 200-mg doses ingested at least 4 hours apart) in addition to the usual menu. The 10-gm dose of L-tyrosine was fed during this period also.

The amount of *l*-ascorbic acid in urine was determined by the method of Evelyn, Molloy and Rosen ('38). The total excretion of hydroxyphenyl compounds, designated as "tyrosyl" compounds, was measured by the method of Medes ('32), which was adapted to the Evelyn colorimeter. The reducing power of the urine was determined as hydroquinone equivalents according to the method of Briggs ('22) adapted to the Evelyn colorimeter. This method measures *p*-hydroxyphenylpyruvic acid, but it is not specific and will also measure homogentisic acid, ascorbic acid, and other reducing sub-

stances. The method of Peters ('42) was used to determine the creatinine content of the urine.

Determinations of ascorbic acid in serum and white cells were made according to the methods devised by Bessey and Lowry and described by Gyorgy ('50) and outlined in Northeast Regional Publication No. 5 ('51).

The method of Roe and Kuether ('43) was used to determine the total ascorbic acid content of the diet after the vitamin had been extracted from the foods by the method of Loeffler and Ponting ('42).

RESULTS

Blood

The average serum and white cell values of ascorbic acid for the 10 subjects during ascorbic acid depletion and repletion are given in table 1. After one week of the low ascorbic acid diet, the serum values had fallen approximately 50%, from 1.1 ± 0.048 to 0.6 ± 0.048 mg %. After 31 days of depletion, the average ascorbic acid level had fallen another 50%, to 0.3 ± 0.039 mg %, and it remained relatively con-

TABLE 1

Average levels of ascorbic acid in the blood and urine, and of "tyrosyl" compounds and hydroquinone equivalents in the urine, of 10 human subjects during ascorbic acid depletion and repletion

DAYS ON DIET	BLOOD ASCORBIC ACID		URINE		
	Serum	White cells	*l*-ascorbic acid	"tyrosyl" compounds	Hydroquinone equivalents
	mg %	*mg %*	*mg/24 hours*	*mg/24 hours*	*mg/24 hours*
Pre-diet	1.1 ± 0.048 [1]				
7 through 12	0.6 ± 0.048	17.1 ± 1.55	5.2 ± 1.0	420 ± 59	110 ± 42
31 through 36	0.3 ± 0.039	13.0 ± 0.92	5.2 ± 0.7	521 ± 91	160 ± 19
54 through 60	0.3 ± 0.047	10.9 ± 0.61	4.8 ± 0.9	482 ± 71	167 ± 17
73 through 78	0.2 ± 0.033	11.5 ± 0.32	4.4 ± 0.5	465 ± 61	201 ± 30
79 through 82 [2]	2.3 ± 0.33	33.4 ± 1.91	165 ± 64	431 ± 61	[3]

[1] Mean ± standard error.

[2] Repletion period; each subject received 800 mg of *l*-ascorbic acid per 24 hours.

[3] Ascorbic acid interferes with this determination.

stant during the remainder of the depletion period. White cell ascorbic acid fell less rapidly. At the end of the first week the white cells contained 17.1 ± 1.55 mg % of ascorbic acid. At the end of one month of depletion there was a decrease of approximately 23% in the cell content. The total decrease of ascorbic acid in the white cells for the 78-day deficiency period was approximately 33%, from 17.1 to 11.5 mg %. The values of 0.2 ± 0.033 and 11.5 ± 0.32 mg % for serum and white cells, respectively, found at the end of 78 days, are almost identical with those of Lowry et al. ('46), who reported values of 0.18 ± 0.01 and 11.9 ± 0.4 mg % at the end of 8 months of depletion.

Of the 23 instances when serum values were less than 0.4 mg % only one white cell value was greater than 14 mg % (fig. 1). No value for white cells under 14 mg % was found when serum values were greater than 0.4 mg %. Both high and low white cell values occurred at a serum level of 0.4 mg %. These are lower white cell values relative to serum values than those reported by Lowry et al. ('46).

Urine

The urinary excretion of ascorbic acid remained low during ascorbic acid depletion (table 1). "Tyrosyl" compound excretion increased from an average of 420 ± 59 mg in the first metabolic period to 521 ± 91 mg per 24 hours during the second period. There was relatively little change in the total amounts of the compound excreted per period as the subjects became more depleted in ascorbic acid, but there was a variation in the daily excretion of "tyrosyl" compounds. The highest daily excretion of "tyrosyl" compounds by a subject was 1,406 mg; the following day the subject excreted less than 1,000 mg. The values found in this study would not be considered indicative of abnormal tyrosine metabolism on the basis of the work of Rogers and Gardner ('49).

There was a trend toward an increase in the reducing power of the urine as the subjects became more deficient in vitamin C. The reducing power of the urine, expressed as

hydroquinone equivalents, increased from an average of 110 ± 42 mg in the first balance period to 160 ± 19 mg per 24 hours in the second balance period (table 1). There was no important change in the total amount excreted in the third

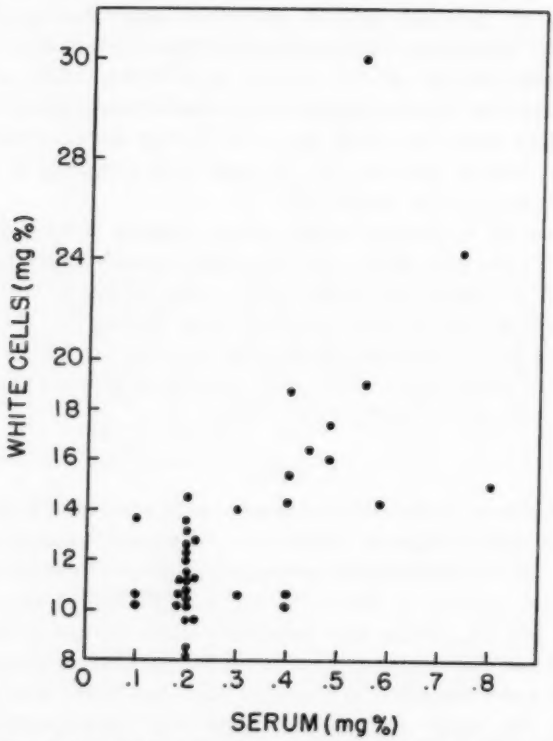

Fig. 1 Comparison of the ascorbic acid concentrations in serum and white blood cells of 10 subjects during depletion of ascorbic acid.

period. During the 4th period the average excretion of hydroquinone equivalents increased to 201 ± 30 mg per 24 hours. The daily excretion of these compounds showed a progressive increase during the 4th metabolic period until the third day. The excretion decreased slightly the next two days and in-

creased again on the last day. Because ascorbic acid contributes to the reducing power of the urine, it was not possible to ascertain whether the hydroquinone equivalents decreased during the subsequent realimentation period. The greatest excretion of reducing substances by any one subject, 706 mg during the 4th period, is above the values found for normal subjects by Rogers and Gardner ('49). The average values reported in table 1, however, are well within the normal range.

Ascorbic acid repletion

All subjects achieved normal values for serum and white cell vitamin C when they were given 800 mg of the vitamin daily for 4 days (table 1). All but two subjects, D.B. and M.G., showed evidence of tissue saturation at the end of the 4th day, as indicated by urinary excretion of approximately 50% of the repletion dose (table 2). The 8 subjects considered satu-

TABLE 2

A 4-day ascorbic acid repletion study on 10 human subjects maintained for 78 days on a low ascorbic acid intake (7 mg or less/day) [1]

SUBJECTS	Day	URINARY ASCORBIC ACID				ASCORBIC ACID RETAINED	WHITE CELL ASCORBIC ACID	
		1	2	3	4		Pre-repl.	Post-repl.
		mg/24 hours				*mg*	*mg %*	*mg %*
D.B.[2]		12.5	218	311	290	635 [3]	13.2	30.5
R.B.C.		3.3	10	277	487	1,171	12.0	40.0
R.C.		4.0	7	170	482	1,265	11.4	31.0
B.E.		9.0	15	101	434	1,177	9.6	29.2
B.G.		4.0	46	417	474	955	10.2	26.5
M.G.		8.0	9	8	105	290 [3]	10.8	35.4
R.H.		6.0	8	76	370	1,020	10.8	31.1
B.L.[4]		5.0	6	38	394	1,133	10.8	29.2
R.L.		3.0	6	116	389	1,042	13.2	33.8
R.R.		4.0	116	529	626	1,231	12.6	47.1
				average		1,120		

[1] Unless noted otherwise, each subject was given 800 mg *l*-ascorbic acid/day for 4 days.

[2] Subject started on repletion one day earlier than others, given 600 mg *l*-ascorbic acid, excreted 4 mg.

[3] Not included in average values.

[4] Received 600 mg *l*-ascorbic acid on second day.

rated retained from 0.966 to 1.265 gm of ascorbic acid over the 4-day period. The retention each day was calculated by subtracting the ascorbic acid excreted that day from the amount excreted at a time when no retention was taking place; e.g., at saturation (Lowry et al., '46).

Subjective symptoms

At the end of 60 days of deficiency, two subjects reported bleeding gums and many of the subjects exhibited increased irritability and complained of undue fatigue. No subject reported having an abnormally high number of respiratory infections. After 76 days of depletion one subject, D.B., had severe skin outbreaks similar to hives and developed small petechiae when these eruptions were scratched. The skin outbreak disappeared after this subject received 600 mg of ascorbic acid on the 77th day and 800 mg daily on each of the succeeding 4 days. It is interesting to note that this same subject was not considered saturated at the end of the experiment, although she had received 3.8 gm of ascorbic acid orally by this time (table 2).

Although the above symptoms were encountered while the subjects were ingesting the diet low in ascorbic acid, the authors do not wish to imply that a cause and effect relationship exists. The symptoms are merely noted as part of the observations made.

DISCUSSION

The ingestion of a diet containing 7 mg or less of ascorbic acid per day by 10 human subjects for 78 days resulted in a lowering of the serum and white cell ascorbic acid content. After 31 days on the deficient diet, the serum ascorbic acid remained at essentially the same low level. White cell ascorbic acid reached its lowest value after the 54th day on the diet.

Similar to the results of Lowry et al. ('46), there seemed to be a relationship between ascorbic acid concentration in the serum and white cells. The present data indicate that with

a serum value *greater* than 0.4 mg %, the subject has a white cell ascorbic acid content of 14 mg % or more. When the serum value is less than 0.4 mg % the cell value is probably below 14 mg % and the adequacy of the ascorbic acid nutriture of the person might be questioned. Thus it appears that some evaluation of past ascorbic acid nutriture may be made on the basis of serum values alone, if the individual's intake of the vitamin has not varied drastically.

During the 78-day period of ascorbic acid deficiency, the only evidence that might be interpreted as being indicative of abnormal tyrosine metabolism was the increased excretion of reducing substances noticed during the 4th period. These values, however, are still within the normal ranges reported by Rogers and Gardner ('49). It is possible that our subjects never became depleted enough in ascorbic acid to manifest abnormal excretion values, although it is known that guinea pigs and infants respond early in ascorbic acid depletion to tyrosine administration.

The work of Levine, Marples and Gordon ('41) indicates that the dosage of tyrosine used is important in eliciting an abnormal response. Rogers and Gardner ('49) gave their scorbutic subjects 20 gm of tyrosine per day. Our subjects received 10 gm per day, or approximately 0.15 gm per kilogram of body weight. While this amount is low when compared with that used by other experimenters, it represents nearly two and one-half times the amount of protein-tyrosine found in a normal diet containing 100 gm of protein (Steele et al., '50). In view of the capability of intestinal organisms to decarboxylate tyrosine and form a toxic amine, it seemed inadvisable to give a higher dose.

SUMMARY

Ten adult humans were fed a diet containing 7 mg or less of vitamin C for 78 days, and the urinary excretion of "tyrosyl" and reducing compounds, and the ascorbic acid level of serum and white cells were determined.

Average serum and white cell ascorbic acid values fell to 0.3 and 13 mg %, respectively, at the end of 31 days of depletion. After 78 days they averaged 0.2 and 11.5 mg %, respectively. These levels returned to normal when the subjects were repleted with 800 mg of ascorbic acid daily for 4 days.

Of the 23 instances when serum values were less than 0.4 mg % only one white cell value was greater than 14 mg %. When serum values greater than 0.4 mg % were encountered (8 cases), no values under 14 mg % were found for white cells.

On the basis of the results of this study, low blood serum ascorbic acid values (less than 0.4 mg %) would indicate a past history of poor ascorbic acid nutriture and an inadequate or deficient intake of this vitamin.

There was an increase in the reducing power of the urine as the subjects became more depleted of ascorbic acid, but the urinary excretion of "tyrosyl" compounds and of reducing substances did not reach abnormal levels during the depletion period, even though 10 gm of free tyrosine were given orally to each subject during the 6 days of each metabolic period.

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PRODUCTION OF MULTIPLE CONGENITAL ABNORMALITIES IN YOUNG BY MATERNAL PTEROYLGLUTAMIC ACID DEFICIENCY DURING GESTATION [1]

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THIRTEEN FIGURES

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Previous studies have demonstrated that when succinylsulfathiazole (SST) is incorporated in a purified diet lacking pteroylglutamic acid (PGA), the vitamin is essential for reproduction in the rat (Nelson and Evans, '47, '49). Such a vitamin deficiency was not pronounced in its effects, as shown by the necessary duration of deficiency required to disturb reproduction, and even under these conditions there were only 26% resorptions. However, the addition of 0.5% of a "crude" PGA antagonist (x-methyl-PGA) produced an immediate effect, so that resorption invariably occurred when rats were placed on such a diet the day of breeding (Nelson and Evans, '49). Macroscopic studies of implantation sites and fetuses indicated that under such conditions the death of the fetus occurred on or before the 11th day of gestation (unpublished data). The present communication reports:

[1] Presented in part before the 63rd meeting of the American Association of Anatomists, April 1950 (Asling and Nelson, '50; Nelson, Asling and Evans '50) and at the autumn meeting of the National Academy of Sciences, November 1951 (Evans, Nelson and Asling, '51). This research was aided by grants from the Board of Research and the Department of Agriculture of the University of California and the Roche Anniversary Foundation.

(1) the occurrence of resorptions when the deficiency is instituted as late as 9 days after breeding; and (2) the production of multiple congenital abnormalities in the young when the deficiency is instituted 10 to 13 days after breeding.

EXPERIMENTAL

Normal female rats (Long-Evans strain), three to 4 months of age, were bred with normal males and placed on the purified PGA-deficient diet containing SST and x-methyl-PGA at various times during gestation. Groups of animals were started 7, 9, 10, 11, 12, 13, and 15 days after breeding.[2] Vaginal smears were examined daily during gestation for the presence of erythrocytes, the sign that implantation has occurred; all rats were weighed at regular intervals. The animals were kept on screens until the day before littering was expected to occur. All rats were autopsied a few hours after parturition or the day before expected parturition (day 21). All fetuses were carefully examined for macroscopic abnormalities and the uterus was checked for the presence of normal or resorbing implantation sites.

The PGA-deficient diet containing the antagonist was the same as that used previously (Nelson and Evans, '49). It consisted of 24% alcohol-extracted casein, 62.5% sucrose, 8% hydrogenated cottonseed oil,[3] 4% salts,[4] 1% SST, and 0.5% x-methyl-PGA. Crystalline vitamins per kilogram diet were: 300 μg *d*-biotin, 5 mg methyl-1,4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg *p*-aminobenzoic acid, 20 mg nicotinic acid, 50 mg *d*-calcium

[2] Many authors differ in timing the days of gestation. In this laboratory the day of finding sperm is called the day of breeding and is considered to be day zero. Wilson and Karr ('51) have recently pointed out that the morning of finding sperm probably represents a fair approximation of the mean time of fertilization and they have, therefore, used this system of timing in their studies on the effects of irradiation. All previous papers by Warkany and by Wilson on congenital abnormalities used a different system of timing, by designating the day of finding sperm as day one.

[3] Crisco or Primex.

[4] Salts 4 of Hegsted et al. ('41).

pantothenate, 400 mg inositol, and 1.0 gm choline chloride. Additional groups of animals received high levels of ascorbic acid and vitamin B_{12}, separately or combined. All rats received weekly a fat-soluble vitamin mixture containing 800 U.S.P. units vitamin A, 115 chick units of vitamin D, 6 mg synthetic alpha-tocopherol, and 650 mg corn oil.[5] The "crude" PGA-antagonist (x-methyl-PGA) used was that described by Franklin et al. ('47).

Control animals maintained on the identical diet supplemented with a high level of the synthetic vitamin, 50.5 mg synthetic PGA per kilogram diet, showed no impairment of reproductive performance and no macroscopic or microscopic abnormalities in the young.

RESULTS

Table 1 summarizes the reproductive performances of rats placed on the PGA-deficient diet 7 to 15 days after breeding and maintained thus throughout the remainder of gestation. The data for rats placed on the deficient diet the day of breeding are included for comparison. The groups averaged 102 to 119 days of age and 212 to 226 gm in body weight on the day of breeding. All groups showed implantation, with a normal number of implantation sites per rat (9 to 11) and the normal appearance of vaginal erythrocytes on the 13th day of gestation. The maternal weight gain during gestation increased from 21 gm when the deficiency was instituted on the day of breeding up to the normal maximum of 100 gm or more when the deficiency was instituted 12 days or more after breeding. All bred rats were normal in appearance and in good condition throughout gestation. In particular, no severe anemia, leukopenia, or granulocytopenia was noted.

Under the experimental conditions, instituting the deficiency 7 or even as late as 9 days after breeding invariably results in 100% resorptions (table 1). In marked contrast are the results obtained when the deficiency is instituted only one day later, day 10. Forty per cent of the animals litter, although

[5] Mazola.

TABLE 1

Occurrence of resorption or abnormal young when mothers are submitted to PGA-deficiency during gestation

PERIOD OF DEFICIENCY (days of gestation)	NO. OF RATS BRED	WT. CHANGE DURING GESTATION	RESORPTIONS	LITTERS	IMPLANTATION SITES PER RAT		YOUNG		
					Total [1]	No. normal	Wt.	Dead	Abnormal
		gm	%	%			*gm*	%	%
1–21	47	+ 21	100	0	10.5	0		None	
7–21	20	+ 46	100	0	9.9	0		None	
9–21	22	+ 61	100	0	9.6	0		None	
10–21	22	+ 70	59	41	11.0	3.0	3.8	100	100
11–21	47	+ 90	4	96	10.2	7.8	4.0	100	95
12–21	23	+ 110	0	100	10.0	9.2	4.5	97	65
13–21	34	+ 100	0	100	10.1	9.5	4.3	56	30
15–21	20	+ 106	0	100	10.3	9.6	5.3	6	0
				PGA controls [2]					
1–21	21	+ 11[illegible]	0	100	11.3	10.6	5.6	4	0
10–21	21	+ 112	0	100	10.0	8.9	5.7	2	0
11–21									

[1] The *total* number of implantation sites per rat equals the number of *normal* sites with living young plus the number of *resorbing* sites; all sites were checked by macroscopic examination at autopsy.

[2] Animals fed the identical diet supplemented with 50.5 mg synthetic peteroylglutamic acid per kilogram.

the number of young per litter and their weight at birth are markedly decreased. Delaying the deficiency until day 11 results in practically normal littering (96% litters), although the weight of the young at birth and sometimes the number of young per litter are decreased. Instituting the deficiency still later in gestation, i.e., day 12, 13, and 15, results in 100% litters with no decrease in the number of young per litter, although the birth weight of the young was less than 5 gm except for the group started on day 15.

TABLE 2

Incidence of macroscopic abnormalities encountered in young from mothers submitted to PGA-deficiency during gestation

PERIOD OF DEFICIENCY (days of gestation)	NO. OF YOUNG	ABNORMAL YOUNG	EDEMA	CLEFT PALATE	SYNDACTYLISM		OTHER ABNORMALITIES
					Fore-paws	Hind-paws	
		%	%	%	%	%	
10–21	39	100	100	100	65	70	Kidneys, lungs
11–21	126	95	95	92	25	50	Kidneys, lungs
12–21	109	65	90	2	0	0	Kidneys
13–21	70	30	slight	0	0	0	Kidneys
15–21	96	0					

Description of abnormal young

The young produced by the mothers given the deficient diet on days 10 to 21 or 11 to 21 are very unusual in appearance and exhibit multiple congenital abnormalities (figs. 1 and 2, tables 1 and 2). In the first group of animals studied, all young were either dead when examined or missing. This prompted examination of the next series of mothers on the day before parturition, day 21. These 21-day-old fetuses were apparently living, as judged by a beating heart and reflex movements, but the lungs did not expand. Ninety-five to 100% of these fetuses were markedly abnormal macroscopically and were characterized by marked edema, anemia, and multiple skeletal and visceral abnormalities. The edema (figs. 1, 2, 5 through 8), which was primarily subcutaneous, resulted in swollen, misshapen, translucent bodies, in some

of which were whitish areas dorsally. The anemia was obvious macroscopically. Hematological studies revealed hemoglobin values as low as 1 to 3 gm per 100 ml and RBC counts of 250,000 to 500,000, in comparison with control values of 10 to 13 gm hemoglobin and two to three million RBC. Other abnormalities obvious on external inspection included cleft palate, marked retardation in facial development, brachydactylism or syndactylism, and short or curled tails. When the animals were cleared and stained with alizarin red (figs. 3 and

TABLE 3

Effect of maternal PGA deficiency, days 11 to 21, on body and organ weights of 21-day-old fetuses[1]

	CONTROLS	PGA-DEFICIENT	% of controls
	mg	*mg*	
Body weight	5,500	3,200	58
Heart	25	18	70
Adrenals	2.7	1.7	63
Testes	4.6	2.7	59
Eyeballs	24	14	57
Brain	215	105	49
Liver	395	186	47
Stomach	38	11	30
Spleen	11	2.3	21
Intestines	152	30	19
Thymus	8	1.5	19
Lungs	108	18	16
Kidneys	49	5	10

[1] These averages are based on 12 control and 14 deficient fetuses.

4), skeletal abnormalities associated with these were revealed and, in addition, retardation in skeletal maturation, deformed or misshapen bones, and the absence of certain ossification centers. Virtually no part of the skeleton escaped damage. In addition, some rats showed alizarin-red staining of the kidneys, the aorta, and the dorsal whitish areas. These skeletal changes are reported in detail elsewhere (Asling, Nelson and Evans, '52).

At autopsy all organs of these fetuses were decreased in weight in comparison with those of the controls. Table 3

reveals that the organs most markedly affected were: the kidneys (10% of normal weight), the lungs (16%), and the thymus, intestines, spleen and stomach (20 to 30% of normal weight). All other organs were 50 to 70% of normal weight and in proportion to the decreased body weight, which was 58% of normal. Macroscopically, the kidneys were extremely small in size, pale, and practically white in color, with occasional cystic pelves. The lungs were also extremely small in size and non-expanded.

Histological studies (to be reported later) revealed a marked retardation in development of kidneys, lungs and skin and a less marked delay in development of practically all other organs and tissues. The lungs were underdeveloped and non-expanded but showed irregular cystic dilatation of many bronchioles (figs. 5 through 8). Both epidermis and dermis were thinner and less differentiated, while the tela subcutanea was tremendously expanded (figs. 5 through 8) and in it were widely scattered primitive connective tissue cells. The distension of this area was responsible for the swollen and misshapen appearance of the fetus (figs. 2 and 6). In the kidneys (figs. 9 through 11) the renal corpuscles and convoluted tubules were markedly underdeveloped, whereas the collecting ducts usually showed greater development and might even appear almost normal. Few connections between the secretory and excretory duct systems could be observed. Occasionally renal corpuscles, but more often the pelvis of the kidney, were markedly dilated and cystic. The eyes, likewise, exhibited retarded development and, more important, a high incidence of Morgagnian-type cataract (degeneration with vacuolization of the lens fibers; figs. 12 and 13).

Timing of the deficiency

The incidence and type of abnormalities varied with the time of instituting the deficiency (tables 1 and 2). Practically all young from mothers placed on the deficient diet on days 10

to 21 [6] or 11 to 21 showed some or all of these abnormalities. Marked edema, cleft palate and visceral abnormalities were present in 90 to 100% of the abnormal young. Although all abnormal young showed some skeletal defects other than cleft palate, syndactylism was higher in days 10 to 21 than in the 11 to 21 group. The hind paws were affected more frequently than the forepaws in the latter group. In contrast, only 65% of the young from mothers placed on the deficient diet one day later, day 12, showed abnormalities, and these were usually less marked. A variable amount of edema and some decrease in kidney size and development were present in 90% of the abnormal young of this group. Only 2% of these young exhibited cleft palates and no syndactylism was observed. Delaying the deficiency one more day, to day 13, resulted in only 30% macroscopically abnormal young; a slight edema was the principal abnormality, although the kidneys did not always attain normality in size. When the deficiency was instituted during days 15 to 21, no abnormal young were observed.

Additional experiments have demonstrated that the timing of the deficiency is the primary factor in abnormal fetal development and mortality, rather than the total amount of PGA-antagonist ingested. The amount of antagonist ingested can be calculated from the level of antagonist in the diet and the duration of the deficiency, assuming an average daily food intake of 16 gm. (This deficiency has only slight effects on food consumption even when instituted on the day of breeding; Nelson and Evans, '49.) The group deficient days 9 to 21, receiving the usual antagonist level (0.5%), consumed approximately 960 mg of PGA-antagonist and showed 100% resorptions. In contrast, a group deficient days 11 to 21, receiving a higher antagonist level (0.6%) which resulted in ingestion of approximately the same amount, 960 mg PGA-antagonist, showed only 17% resorptions. Another group deficient days 10 to 21, receiving a lower level (0.4%), con-

[6] Additional abnormalities of the urogenital and cardiovascular systems were observed in the group made deficient on days 10 to 21; these will be reported later.

sumed an estimated 700 mg antagonist, which resulted in 38% litters, with 100% of the young exhibiting marked abnormalities; while the group deficient days 12 to 21, receiving the usual level (0.5%), consumed an estimated 720 mg antagonist, which resulted in 100% litters with only 65% of the young showing abnormalities, and these were less severe.

A similar gradation was shown in the percentage of dead young from these groups, one-third to one-half of which were allowed to litter. Instituting the deficiency on day 10 or 11 resulted in 100% and on day 12 in 97% dead young. In contrast, delaying the deficiency to day 13 resulted in a marked decrease to 56% dead young, and a further delay to day 15 in only 6% dead young, a close approximation to the maximum of 2% dead young for stock animals or 2 to 4% dead young for controls receiving high levels of the synthetic vitamin.

DISCUSSION

The data presented show the severity of pteroylglutamic acid deficiency as a teratogenic agent for the rat. Multiple congenital abnormalities in extremely high incidence, 95%, characterized the young from mothers placed on the deficiency regimen 11 days after breeding. The observed abnormalities, i.e., anemia, edema, skeletal malformations such as cleft palate and syndactylism, retarded visceral development which was especially noticeable in the kidneys and lungs, and Morgagnian-type cataracts, are in some respects similar to or identical with congenital abnormalities reported for other vitamin deficiencies in the rat, but in other respects they are unlike any malformations previously reported.

The skeletal malformations we have observed in this study are similar to but more extensive than those reported for riboflavin deficiency in the rat (Warkany and Nelson, '41). The timing for production of these skeletal anomalies is, however, similar for both deficiencies, i.e., we have found the critical time for starting the PGA-deficiency to be between the 11th and 12th days whereas Warkany et al. ('42) found that the critical time for vitamin (B_2) supplementation of the

diet to be between the 12th and 13th days.[7] It is a reasonable assumption that the institution of the PGA-deficient diet will require at least one day to affect the fetus. The edema observed is similar but apparently much more extensive than that reported for deficiencies of either vitamin A (Wilson and Warkany, '48) or pantothenic acid (Boisselot, '49, '51). The retardation of lung development is similar to that reported for vitamin A deficiency (Wilson and Warkany, '48), although no cystic enlargement was observed in vitamin A deficiency. The retardation of kidney development is macroscopically similar to hypoplasia of the kidney encountered in the same studies of vitamin A deficiency but differs in affecting primarily the renal corpuscles and convoluted tubules (the secretory portion) instead of the collecting tubules or excretory portion of the kidney. Morgagnian-type cataracts of the eyes have not been reported previously for any vitamin deficiency, adult or fetal, in the rat though other congenital abnormalities of the eyes are associated with vitamin A deficiency (Warkany and Schraffenberger, '46) and pantothenic acid deficiency (Boisselot, '49, '51). The marked anemia observed is similar to that produced by PGA deficiency in the adult rat and may, therefore, be classified as a sign of fetal PGA-deficiency, just as keratinization of the fetal urogenital tract is a sign of fetal vitamin A deficiency (Wilson and Warkany, '47) and fetal cataract of fetal tryptophan deficiency (Pike, '51). Giroud and Boisselot ('51) have mentioned the occurrence of anemia in young from PGA-deficient mothers.

Previous reports have been made on congenital abnormalities, other than those reported in this study, resulting from maternal PGA-deficiency in the rat and other animals. Richardson and Hogan ('46) reported a low incidence (2%) of hydrocephalus in young from rats maintained for long periods on purified diets. Further studies have raised the incidence to more than 20% and have indicated that both vitamin B_{12} and PGA are concerned (O'Dell et al., '51). A similar pos-

[7] Warkany's timing is corrected in accordance with identifying the day of finding sperm as day zero, corresponding with the timing used in this paper.

sible double deficiency is that reported by Ross et al. ('44) for swine maintained on vegetable protein diets; skeletal anomalies such as syndactylism, talipes and dyostosis were noted together with edema, eye abnormalities or blindness, and paralysis agitans. By injections of various PGA-antagonists, Karnofsky et al. ('49) produced vascular abnormalities, absence of feathers, limb and beak abnormalities, and exteriorization of the viscera in chick embryos. Likewise Sunde et al. ('50) have found that maternal PGA deficiency in hens resulted in perosis, deformed mandibles, parrot beak, or fetal death for chicks. Recently Giroud and Boisselot ('51) have briefly reported the occurrence of multiple congenital abnormalities in young from rats maintained on a PGA-deficient diet containing SST. Anemia, hemorrhage, retinal colobomas, harelip with cleft palate, oblique facial fissures, atrophy of the nasal fossae, ectocardia and gastroschisis were observed. These abnormalities are similar to those we have observed with a transitory maternal PGA-deficiency early in gestation (data to be published).

The similarity of many of the congenital abnormalities observed in this study of maternal PGA-deficiency to those reported for other teratogenic vitamin deficiencies in the rat raises the question of the specificity of the vitamin deficiency produced but, even more important, the question of the specificity of the teratogenic action of any vitamin deficiency. The importance of timing the deficiency in regard to fetal death, abnormal young, or normal young, and especially the fact that the timing for the skeletal abnormalities observed in this study was similar to that observed for similar anomalies produced by riboflavin deficiency, would seem to indicate that the timing of the deficiency is of more importance than the specific nature of the deficiency. Stockard ('21) has emphasized the importance of timing in experimental teratology. In regard to this study of PGA-deficiency, the action of the PGA-antagonist (x-methyl-PGA) can be reversed only by high levels of PGA; in addition, high levels of ascorbic acid or vitamin B_{12} or both had no effect.

SUMMARY

Under the experimental conditions employed, instituting a deficiency of pteroylglutamic acid in the rat as late as 9 days after breeding invariably resulted in fetal death (resorption), whereas delaying the deficiency two days to 11 days after breeding resulted in 95% of the animals littering young with multiple congenital abnormalities. These young were characterized by a marked edema and anemia, multiple skeletal abnormalities such as cleft palate and syndactylism, retarded development of the viscera, especially kidneys and lungs, and Morgagnian-type cataracts of the eyes.

Instituting the deficiency between day 9 and day 11, i.e., 10 days after breeding, resulted in approximately 40% of the animals producing litters, consisting of a few markedly abnormal young. In contrast, delaying the deficiency to 12 or 13 days after breeding resulted in 100% litters, with the young having milder degrees of edema and visceral retardation and practically no skeletal abnormalities. Normal young resulted when the deficiency was not started until 15 days after breeding.

The similarity of some of the abnormalities observed to those previously reported for other vitamin deficiencies in the rat is discussed.

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PLATE 1

EXPLANATION OF FIGURES

Comparison of external form and skeletal structure of 21-day-old fetuses from mothers maintained on the PGA-control or deficient diet from the 11th to the 21st day of gestation.

1 Control fetus ($\frac{1}{2}$ natural size).

2 Deficient fetus ($\frac{1}{2}$ natural size). Note edema, translucence and whitish plaques on dorsum.

3 Control fetus, cleared and stained with alizarin red S. × 3.2.

4 Deficient fetus. × 3.2. Note general stunting, disproportionately short mandible, reduced number of ossification centers (as in paws), deformed bones (scapula, ribs, radius, ulna, and tibia) and defective vertebral formation.

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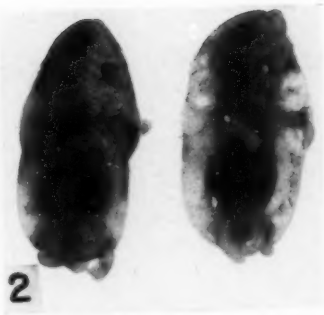
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PLATE 2

EXPLANATION OF FIGURES

Histologic sections of 21-day-old fetuses from mothers maintained on the PGA-control or deficient diet from the 11th to the 21st day of gestation. Hematoxylin and phloxine B stain, 10 μ thickness.

5 Sagittal section of control fetus. × 2.4.

6 Sagittal section of deficient fetus. × 2.4. Note thinner skin, edema of subcutaneous tissue and reduced size of all viscera. The lung is non-expanded but shows irregular cystic dilatations.

7 Transverse section of control fetus through upper part of thorax. × 4.2. Arrow indicates normally expanded lung.

8 Transverse section of deficient fetus through same area. × 4.2. Arrow indicates non-expanded lung tissue, still in fetal position. Note also the edema which extends into the extremities.

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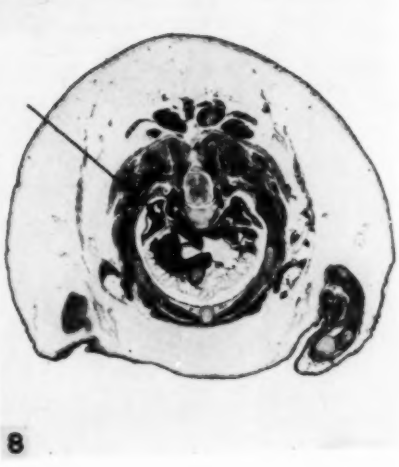

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PLATE 3

EXPLANATION OF FIGURES

Histologic sections through kidneys and eyes of 21-day-old fetuses from mothers maintained on the PGA-control or deficient diet from the 11th to the 21st day of gestation. Hematoxylin and phloxine B stain, 8 μ (kidneys) to 10 μ (eyes) thickness.

9 Kidney from control fetus. × 21. Renal corpuscles are well-developed and majority have established connections with excretory ducts; kidney is ready to function.

10 Kidneys and adrenals from deficient fetus. × 21. Note marked reduction in kidney size, scarcely exceeding that of the adrenals, and retardation in development.

11 Higher magnification (× 55) of right kidney seen in figure 10. This shows the marked reduction of glomerular development and lack of connections of renal corpuscles with excretory ducts. Only a few collecting ducts are seen in the medulla. Note cystic dilatations in convoluted tubules.

12 Eye of control fetus. × 21.

13 Eye of deficient fetus. × 21. Note reduction in size of eye and degeneration, with irregular vacuolation of the lens fibers. These changes in the lens are similar to those in congenital Morgagnian cataract observed clinically.

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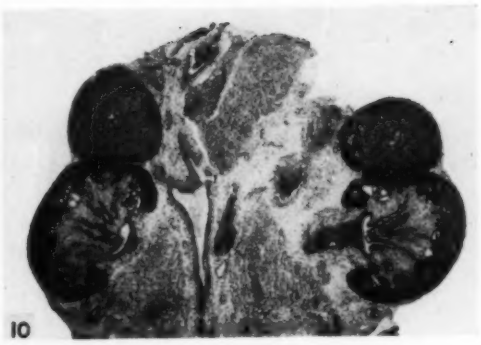
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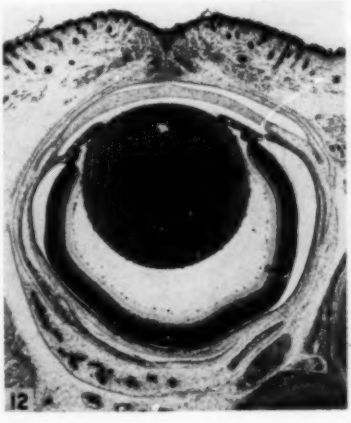
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EFFECT OF THE LEVEL OF FAT IN THE DIET ON THE GROWTH PERFORMANCE OF DOGS[1]

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THREE FIGURES

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Considerable interest has developed in the use of inedible fats in dry dog foods, particularly when such fats are in excess supply. McCay ('49) has stated that fats are well utilized by dogs; however, to our knowledge, a systematic study of the performance of dogs fed graded levels of fat in addition to a basal diet comprised of crude ingredients commonly used in commercial meals has not been reported. Although 10 to 15% fat is commonly added to purified diets for dogs, only 5 to 7% fat is used in commercial dry meals. In view of these considerations, it was of importance to evaluate the effect of adding fat, stabilized with an anti-oxidant, to practical diets on the growth rate, and food and caloric efficiencies of young dogs.

EXPERIMENTAL

Experiment 1

An experimental (basal) ration was formulated with ingredients commonly used in commercial dry dog meals to

[1] Journal Paper 5[illegible] American Meat Institute Foundation.

A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. The contract is being supervised by the Eastern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry.

provide adequate amounts of the known growth factors for the dog as outlined by Michaud and Elvehjem ('44). As a further test of the formulated ration, a commercial dry meal was purchased directly from the manufacturer and compared with the experimental ration. The composition of the experimental ration and the proximate analysis of pooled samples of both rations are given in tables 1 and 2.

TABLE 1

COMPOSITION OF EXPERIMENTAL RATION [1]	AMOUNT
	%
Corn flakes	26.75
Wheat flakes	26.70
Soybean grits (HI-PRO-CON)	19.00
Meat and bone scrap	15.00
Fish meal (Menhaden)	3.00
Wheat germ meal (defatted)	5.00
Dried skim milk	2.50
A and D oil (Nopco xx-2250 U.S.P. units A, 400 A.O.A.C. units D/gm)	0.50
Iodized salt	0.25
Brewers' yeast (non debittered)	0.50
Riboflavin supplement (BY-500)	0.80
	100.00

[1] We are indebted to the Schlitz Brewing Co., Milwaukee, Wisconsin, for supplying the yeast, and to the A. E. Staley Manufacturing Co. for supplying some of the soybean grits used in this study.

TABLE 2

Proximate analysis of rations [1]

ANALYSIS	COMMERCIAL RATION	EXPERIMENTAL RATION
	%	%
Moisture	6.1	6.3
Protein (N × 6.25)	25.7	29.1
Fat (ether extract)	5.9	3.7
Ash	6.9	6.7
Fibre	4.9	2.4

[1] Made by the Service Laboratory, American Meat Institute Foundation.

The added fat (choice white grease — a high grade of inedible pork fat) was stabilized by the addition of butylated-hydroxyanisole 0.02%, citric acid 0.01%, and propyl galate 0.005% (Kraybill et al., '49; Dugan et al., '50) to the melted fat at 80°C.

The rations were made up 60 days prior to feeding to approximate the shelf life of commercial meals and to investigate the development of rancidity during storage. Separate studies are being conducted on the effect of different anti-oxidant combinations on the stability of greases added to feeds (Neumer and Dugan, '52).

Registered cocker spaniel pups of mixed sex (average age of 12 weeks) were purchased, treated for intestinal parasites, dipped into a DDT solution for removal of external parasites, injected subcutaneously with anti-canine distemper serum, and individually housed in sterilized metal cages equipped with raised, expanded metal bottoms in a room thermostatically heated to 76°F. The dogs were fed the experimental ration during an initial two-week orientation period during which an infection (controlled through oral administration of antibiotics) occurred among some of the dogs.

At the termination of an additional two-week orientation period, the dogs were arranged into 6 feeding groups as evenly as possible (judged by litter, weight, sex and body conformation) and fed either the experimental ration (three dogs), experimental ration + 6% stabilized fat (three dogs), experimental ration + 6% stabilized fat with the caloric intake per dog restricted to the average caloric intake per dog for the group fed the experimental ration (4 dogs), experimental ration + 13.5% sucrose (sucrose equal in crude calories to 6% fat) (three dogs), commercial dog meal (three dogs), or commercial dog meal + 6% stabilized fat (4 dogs). The fat and sucrose were added at the expense of the entire ration. All dogs except those calorically restricted were fed ad libitum. All rations were fed dry and all groups were given water ad libitum. The group fed sucrose was included to determine if the effect of feeding fat could be attributed to

factors other than calories and to evaluate further any effect of diluting other nutrients (minerals, vitamins, proteins) associated with the addition of fat or sucrose to the rations.

The dogs were fed the rations for a 10-week period and weighed at weekly intervals. Food consumption records were obtained for each dog during the first through 8th week on experiment. The food and caloric efficiencies were calculated from these data.

Experiment 2

A second experiment was performed using female cocker spaniel pups (12 weeks of age) as the test animals. Female dogs were used in order to obtain reproduction and lactation data when they were fed various levels of fat. The results of the reproduction and lactation studies will be available at a later date. The basal ration, the stabilized fat added to the rations, and the treatment and housing of the dogs were identical with those of the first experiment. All rations were freshly mixed before feeding. Four groups of weanling female cocker spaniel pups were used (7 dogs in each group) and fed either the experimental ration, the experimental ration + 4% stabilized fat, the experimental ration + 8% stabilized fat or the experimental ration + 18% sucrose (sucrose equal in crude calories to 8% fat). The dogs were weighed at weekly intervals over a 10-week period. One dog in the group fed 8% added fat and two dogs fed the sucrose diet died during the first two weeks of the experiment from unknown causes. Food consumption records were obtained from the 4th through 7th week of the experiment. All dogs were fed and given water ad libitum and all rations were fed dry.

RESULTS AND DISCUSSION

The dogs fed the basal diet in experiment 1 averaged 371 gm gained per week, while the dogs fed the commercial ration averaged 334 gm gained per week (table 3). No differences in average food and caloric efficiencies were noted between these groups, indicating that the experimental ration used was

equal to, or better than, the commercial ration. The rates of gain and the food and caloric efficiencies of the groups fed 6% fat in addition to either the basal ration or commercial ration were equal to or slightly better than those for

TABLE 3

Effect of feeding different levels of fat on the rate of gain and the food and caloric efficiency of dogs

RATION	NO. OF DOGS	AVE. INITIAL WT.	AVE. GAIN/WK.	FOOD EFFICIENCY [1]	CALORIC EFFICIENCY [2]
		gm			
Experiment 1					
Commercial ration	3	2,920	334	0.22	6.2
Commercial ration + 6% fat	4	2,490	383	0.24	6.2
Experimental ration	3	3,140	371	0.22	6.1
Experimental ration + 6% fat	3	3,410	443	0.26	6.8
Experimental ration + 6% fat [3]	4	3,150	435	0.24	6.4
Experimental ration + 13.5% sucrose	3	2,990	402	0.22	6.2
Experiment 2					
Experimental ration	7	3,300	339	0.18 ± 0.011 [4]	5.2 ± 0.32 [4]
Experimental ration + 4% fat	7	3,180	360	0.17 ± 0.022	4.5 ± 0.55
Experimental ration + 8% fat	6	3,870	334	0.17 ± 0.026	4.4 ± 0.59
Experimental ration + 18% sucrose	5	3,310	328	0.15 ± 0.011	4.1 ± 0.30

[1] Grams gained per gram food consumed.
[2] Grams gained per 100 crude calories consumed.
[3] Caloric intake restricted to that of the group fed the experimental ration (see text).
[4] Standard error.

the groups fed the rations without added fat (figs. 1 and 2; table 3). The performance of the group fed sucrose was similar to that of the group fed the basal diet. The group fed the experimental ration + 6% fat with the caloric intake restricted to the caloric intake of the group fed the experi-

mental ration did not consume the calories allotted and, therefore, can be considered as having been fed ad libitum. Excellent agreement was found for the average rates of gain, food efficiencies, and caloric efficiencies between the two groups

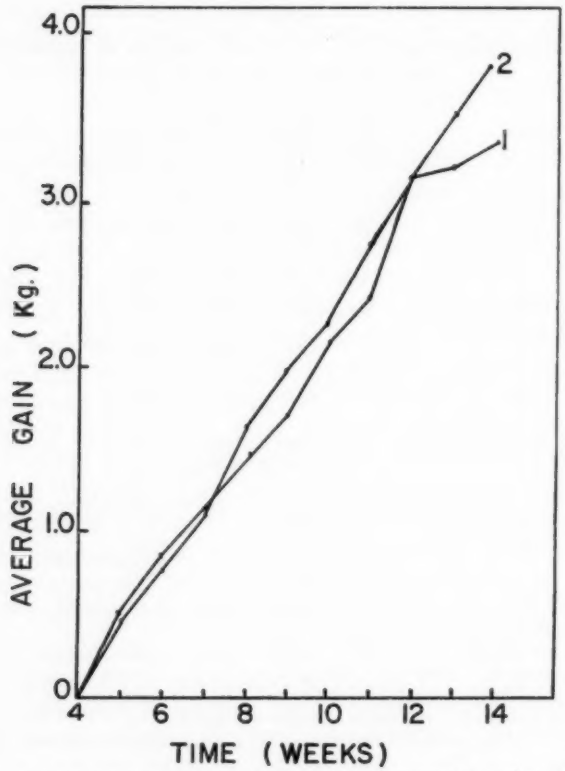

Fig. 1 Average rate of gain of dogs fed the commercial ration (curve 1) or the commercial ration plus 6% fat (curve 2) in experiment 1.

receiving 6% added fat, as is indicated in table 3. Therefore, the rates of gain per week for these two groups are shown as a single curve in figure 2. No differences in general appearance, health, and haircoats were noticed in any of the groups tested.

Organoleptic evaluations of the rations with added fat showed that no detectable rancidity developed during storage for two months at room temperature.

The average gain per week for the various groups used in the second experiment are summarized in figure 3 and the

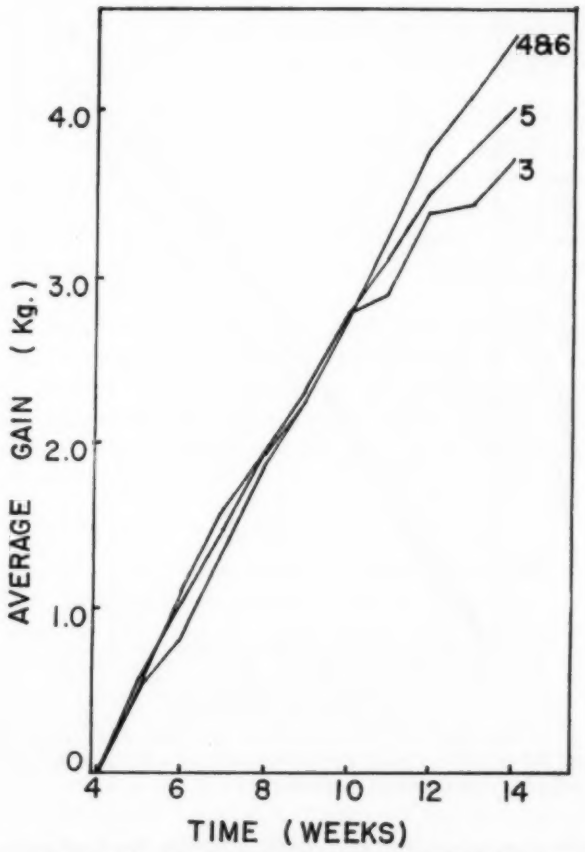

Fig. 2 Average gain per week for dogs fed the basal ration (curve 3) or this ration + 6% fat (composite for calorie-restricted and ad libitum-fed groups, curves 4 and 6 — see text) or the basal ration + 13.5% sucrose (curve 5), experiment 1.

results over a 10-week period for the food and caloric efficiencies are shown in table 3. The results for this experiment confirmed those obtained in the first experiment, in

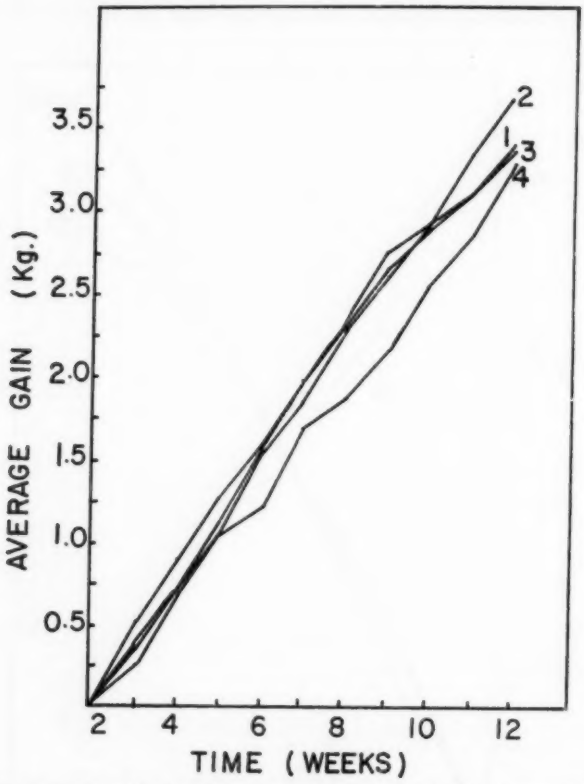

Fig. 3 Average gain for the groups fed the basal diet (curve 1), basal diet + 4% fat (curve 2), basal diet + 8% fat (curve 3), or basal diet + 18% sucrose (curve 4) in the second experiment.

that the average gains per week and the food efficiencies of all the groups tested were quite uniform, although the group receiving 4% added fat showed somewhat higher average gains per week.

Somewhat lower rates of gain were observed in the second experiment than in the first experiment. This variation may have been due to the absence of male dogs and the smaller body conformation of many of the dogs used in this second experiment. The caloric efficiencies for the groups fed added fat or sucrose were somewhat lower than for the basal group in this experiment. Except for the two dogs excluded from the results, the general appearance, haircoat and health of all dogs were excellent.

The results on an over-all basis are in good agreement for the two experiments and indicate that the performance of weanling cocker spaniel pups fed the basal diet plus 4, 6, or 8% fat was equal to or slightly better than that of the dogs fed the basal diets without added fat, based on rates of gain, general appearance, food utilization and the health of the pups.

These results indicate that widening the protein, mineral, and vitamin-to-calorie ratios by the addition of fat did not reduce the rate of gain. Therefore, the protein, mineral and vitamin content of the experimental ration was in excess of the requirements of cocker spaniels during the stage of growth used in these studies.

SUMMARY

The rate of gain of young cocker spaniel pups fed diets comprised of ingredients commonly used in dry meals, with or without added fats (choice, white grease stabilized with antioxidants), was investigated. The rates of gain for a 10-week period, when 4, 6, or 8% fat was added to the basal diet or when 6% fat was added to a commercial meal, were equal or slightly superior to those obtained when the diets without added fat were fed. No significant differences in the food or caloric efficiencies were noted between the groups fed different levels of fat, which indicated that the calories from the fat were well utilized. The performance of groups fed sucrose (equivalent in crude calories to the added fat) was comparable to that of groups fed the basal diet.

It is concluded that 4, 6, or 8% stabilized fat can be successfully added to the experimental ration used in these experiments, as judged by rates of gain, food utilization and the general health of young cocker spaniel pups.

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THE INFLUENCE OF DIETARY FAT AND CARBOHYDRATE ON REPRODUCTION AND LACTATION IN RATS[1]

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The value of dietary fat for successful reproduction and lactation has been established for both natural and purified diets with a number of species (Evans et al., '34; Maynard and Rasmussen, '42; Nelson and Evans, '47a). The latter reported that although growth and reproduction could be satisfactorily maintained in long-term studies with purified diets, the function of lactation involves additional dietary requirements. These workers (Nelson and Evans, '47b) found an inverse relationship between lactation performance and the fat content of the diet that could be corrected by supplementation with liver eluate powder. Sica and Cerecedo ('48), however, maintained that a diet which is adequate in all respects for reproduction will also be adequate for lactation, and proposed the birth weight of the young as the primary criterion for evaluating a diet.

Deuel and co-workers ('47, '50) have more recently investigated the influence of varying amounts and kinds of fat in purified diets. Here the results were less conclusive, indicating that the type of fat in itself exerted little influence on lactation as long as it was present in appreciable amounts. Meyer et al. ('51), however, in a continuation of studies concerning the impairment of fertility and lactation by diets containing pork, were unable to assign a definite

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reason for such an effect. They emphasized the need for basic investigation concerning the influence of the composition of the diet as a whole on the requirements for reproduction and lactation. They found that reduction in the fat content of their pork-containing diets, either by adding sucrose (dilution effect) or by removal of a portion of the fat, resulted in an improvement in fertility and lactation. This finding appears significant in view of evidence to be presented in this paper.

A series of experiments summarized by Swift and Black ('49) revealed the beneficial effects of dietary fat in the reduction of heat production and heat increment and in the increased efficiency of food utilization, producing greater growth with high-fat diets as compared to high-carbohydrate diets of equal energy content. Since these experiments involved specific periods in the lifetime of the rat, it was deemed desirable to extend the work to include comparable observations on reproduction and lactation, which are discussed in the present report, and growth and longevity studies still in progress.

EXPERIMENTAL

The three diets shown in table 1 were prepared at monthly intervals, sampled for analysis, and stored in a refrigerator until fed. The control diet consisted of a finely ground commercial rat ration that had given satisfactory reproduction and lactation for a number of years in the breeding colony.[2] The two experimental diets, one high in carbohydrate and one high in fat, were prepared by mixing the control diet at an 80% level with 20% of corn oil or 20% of sucrose, respectively. The latter two diets were supplemented equally with liver powder, yeast and vitamins to insure optimum levels of known and unknown factors required for reproduction and lactation.

The experimental feeding extended over a period of more than two years. Since it would be impractical to store one

[2] Rockland Farms Rat Diet, complete *(see bottom of next page)*.

lot of feed for such a time, each monthly mixing included all three diets prepared from one batch of the commercial ration. Successive monthly mixes utilized different lots of this ration but were fed to similar numbers of litter-mate animals distributed evenly among the three diets. In this manner any minor change in the composition of the commercial ration as the experiment progressed would influence equally each of the three diet groups.

The experimental animals were obtained by breeding closely related albino rats of the Wistar strain that had reached maturity on the experimental diets as a part of the growth and longevity studies. Approximately 20 males and 20 females were chosen from each of the three diet groups and were mated individually when they reached 120 days of age to produce the first generation of young. (First-litter young of this strain have been observed to be the equal of young from subsequent litters when the dams are allowed to mature to 120 days of age and 230 gm weight before mating.)

Succeeding generations were obtained by retaining a male and female from each litter, feeding the same experimental

ROCKLAND FARMS RAT DIET

Mineral composition
(By chemical determination, %)

Silica (SiO_2)	0.59	Calcium (Ca)	2.13
Chlorine (Cl)	0.96	Phosphorus (P)	0.71
Sodium (Na)	0.88	Sulphur (S)	0.24
Potassium (K)	1.12	Magnesium (Mg)	0.33
	Milligrams per 100 gm		
Manganese (Mn)	6.80	Boron (B)	1.37
Iron (Fe)	37.00	Cobalt (Co)	0.35
Zinc (Zn)	2.00	Iodine (I)	1.20
Copper (Cu)	0.27	Fluorine (F)	1.90

Vitamin content
(By rat bioassay determination, per 100 gm)

Vitamin A	610–680 I.U.	Inositol (free) [a] [b]	61 mg
Vitamin B_1	228–465 μg	Vitamin D	148 U.S.P. units
Riboflavin B_2	538–652 μg	Alpha-tocopherol	7 mg
Pyridoxine B_6	209–275 μg	PABA [b]	78 μg
Choline equiv.	480–492 mg	Pantothenic acid	1989–2568 μg

[a] Mouse bioassay.
[b] Also by microbiological assay.

(By chemical determination, per 100 gm)

Niacin	14.4 mg	Carotene	42–45 mg
Vitamin C	3.60 mg	Choline	152 mg
	Ratios		
Ca:P	3:1	Mn:B_1	14:1
Ca:Mg	6.5:1	NaK	1:1.3

	anti-alopecia inositol diet level	Purified 70%	Stock 30%

Guaranteed analysis

Protein	21.00%	Fat	4.00%	Fibre	6.00%

diets until they reached 120 days of age, and then breeding them with mates from different litters but receiving the same diets. In the third generation the females were rested one month after weaning their young and then rebred to different males to obtain a second litter. Since the data showed no increase in size, weight or number of young from the second

TABLE 1

Composition of diets

	CONTROL	CARBOHYDRATE	FAT
	gm	*gm*	*gm*
Constituents			
Rat ration [1]	100	80	80
Sucrose	..	20	..
Corn oil [2]	..	..	20
Supplements			
(per 100 gm diet)			
Liver, 1–20,[3] gm	..	1.0	1.0
Yeast, brewers', gm	..	0.5	0.5
Yeast, irradiated, gm	..	0.5	0.5
Choline chloride, mg	..	160	160
Alpha-tocopherol acetate, mg	..	5	5
Thiamine hydrochloride, mg	..	0.2	0.2
Analysis			
Energy, Cal./gm	3.910	3.935	5.011
Nitrogen, %	3.77	3.09	3.11
Ether extract, %	4.35	3.38	22.72
Moisture, %	10.33	8.23	7.87

[1] Rockland Farms Rat Diet, complete.
[2] Mazola.
[3] Wilson's 1–20 liver powder.

breeding, these results were averaged with those of the first breeding for this generation. An average of 78 matings for each diet in three generations was thus completed. When pregnant, the females were removed from the screen-bottomed cages and placed in individual cages with wood shavings for nesting. The young were counted, examined for sex, and weighed soon after birth and at three days of age, at which time each litter was reduced to 6 rats, preferably three of

each sex as recommended by Daggs ('35). They were weighed again at 14 days of age and when weaned 21 days after birth. Approximately 350 young in the three generations were raised on each diet. The dams were similarly weighed after parturition, and at three days, 14 days and 21 days. The environmental temperature was maintained throughout the experiment at 27 ± 2°C. with few exceptions.

Food was supplied ad libitum, and although food consumption was determined in the growth and longevity phases, spillage and contamination with wood shavings and excreta in the nesting cages prevented it in the present study. The average daily consumption of the fat, carbohydrate, and control diets by non-pregnant females of like age, weight and breeding as measured in the growth studies was 53.6, 54.7 and 59.8 Cal., respectively.

The methods used for analysis of the composited diet samples for energy, nitrogen, crude fat and moisture were, respectively, the bomb calorimeter, Kjeldahl procedure, Soxhlet ether extraction, and vacuum drying to constant weight over concentrated sulfuric acid.

RESULTS AND DISCUSSION

Since the reproduction and lactation performances compared in this experiment were determined on essentially complete and natural diets where differences were expected to be minimum, every precaution was taken to record all data that might indicate any slight effect. Accordingly, the techniques and measurements proposed by Daggs ('35), Schweigert ('47), Sica and Cerecedo ('48), Mirone et al. ('48), Henderson et al. ('48), Goettsch ('49), Sherman et al. ('49) and others were considered and as many as possible adopted.

Reproduction

If we may define good reproductive performance as the ability to conceive and give birth to large litters of living young of sufficient birth weight to insure survival, then it

will be observed (table 2) that the animals receiving the high carbohydrate diet were in all respects similar to those on the control diet. The rats in the high-fat group, however, in each generation produced fewer numbers of young per litter and young of lighter weight. This result appears even more significant when it has been observed that under normal conditions smaller litters generally consist of heavier-than-average young. While it is true that the protein levels in the fat and carbohydrate diets were somewhat lower than in the control diet (19.4, 19.4 and 23.6%, respectively), the improved quality of the protein from the yeast and liver supplements would tend to minimize this difference. The overall reduction in protein intake on the two experimental diets was not severe enough to limit growth or lactation and it seems unlikely that it contributed to the decreased reproductive performance of the high-fat group, although this possibility cannot be excluded. Certainly the slight differences in caloric intake between the fat and carbohydrate groups would not have been responsible for the significant difference in reproductive performance.

The female young at birth on each diet weighed about ½ gm less than the males and the possibility existed that a shift in sex ratio of the young might have contributed to the apparent dietary effect. This was not found to be the case, however. The numerical ratios of male to female young born were found to be 1.06, 1.01 and 1.02 for the control, carbohydrate and fat diets, respectively. Although the numbers involved were not large, it would appear that the sex ratio was not affected by diet and did not contribute to the differences in birth weight observed between the diets.

The average birth weight of the young in all groups exceeded the minimum range for survival of 5.0 to 5.4 gm (Sica and Cerecedo, '48) and no differences were observed in the number of young surviving to the third day. All three diets would appear to be adequate with respect to specific dietary factors required for reproduction.

TABLE 2

TABLE 2

Summary of reproduction data

DIET	GENERATION	NUMBER OF			NUMBER OF YOUNG BORN			AVERAGE BIRTH WEIGHT OF YOUNG	
		Dams	Matings	Result in litters [1]	Dead	Alive	Alive on 3rd day	Male	Female
								gm	*gm*
Control	1st	17	17	15	2	173	171	6.4	5.9
	2nd	19	19	16	8	144	140	6.6	6.1
	3rd	14	28	22	5	214	209	6.5	6.0
	Total	50	64	53	15	531	520		
	Average per litter				0.3	10.0 ± 0.44 [2]	9.8 ± 0.44	6.5 ± 0.08	6.0 ± 0.07
Carbohydrate	1st	21	21	21	16	206	206	6.5	6.0
	2nd	21	21	19	5	209	209	6.3	5.9
	3rd	18	36	30	14	296	286	6.5	6.1
	Total	60	78	70	35	711	701		
	Average per litter				0.5	10.2 ± 0.37	10.0 ± 0.38	6.4 ± 0.07	6.0 ± 0.07
Fat	1st	23	23	21	12	199	198	6.2	5.8
	2nd	23	23	23	14	203	195	6.3	5.8
	3rd	22	45	43	29	406	391	6.0	5.6
	Total	68	91	87	55	808	784		
	Average per litter				0.6	9.3 [3] ± 0.32	9.0 [3] ± 0.33	6.1 [4] ± 0.08	5.7 [4] ± 0.07

[1] All unsuccessful matings involved two or more trials with fertile males.
[2] Standard error of the mean.
[3] Significantly different at 5% level from carbohydrate group.
[4] Significantly different at 1% level from either control or carbohydrate group.

A somewhat higher proportion of matings were successful in animals receiving the high-fat as compared to the other diets, but when the failures were examined individually, similar numbers of resorptions were noted; 4, 4, and three, respectively, for the control, carbohydrate and fat diets. One female in each of the carbohydrate and fat groups died at parturition and the few remaining failures were considered to be due to female sterility after two or more unsuccessful matings with fertile males.

The average number of days elapsing between mating (as defined by placing a male and female together) and parturition was similar in all diet groups, averaging 27.2, 28.0 and 27.7 days for rats on the control, carbohydrate and fat diets, respectively. The majority of the females gave birth 23 to 28 days after mating, with a few on each diet, however, requiring as many as 50 days. Male fertility was excellent in all diet groups through all generations.

Lactation

Lactation performance was measured primarily by recording the gain in weight of the young during the period from the third to the 14th day, during the time that their sole source of nourishment was obtained by nursing. The data, summarized in table 3, indicate a significant beneficial effect on lactation of the high-carbohydrate diet. The young of each sex in the carbohydrate group gained about 7% more weight than did those on the fat or control diets. This finding extended into the third week of lactation, when the young were gradually consuming increasing amounts of the experimental diets. The average gain in weight during the third week was 14.7 and 13.9, 17.5 and 16.7, and 15.3 and 15.2 gm for the males and females in the control, carbohydrate and fat groups, respectively. At 21 days of age the young of both sexes on the carbohydrate diet were significantly heavier than the other two groups. The difference was in the order of 10%, indicating that the improvement in lactation was ef-

TABLE 3

Summary of lactation data

DIET	GENERATION	NUMBER OF YOUNG [1]	AVERAGE GAIN IN WEIGHT FROM 3RD TO 14TH DAY OF AGE		AVERAGE WEANING WEIGHT AT 21 DAYS OF AGE	
			Male	Female	Male	Female
			gm	*gm*	*gm*	*gm*
Control	1st	90	20.6	19.8	44.3	42.1
	2nd	66	21.9	21.1	46.4	43.9
	3rd	108	19.1	18.9	43.5	41.7
	Total	264				
	Average		20.3 ± 0.43 [2]	19.8 ± 0.39	44.5 ± 0.65	42.4 ± 0.60
Carbohydrate	1st	102	22.6	21.5	49.2	47.1
	2nd	108	21.6	20.9	48.3	46.6
	3rd	156	21.9	21.0	49.0	46.5
	Total	366				
	Average		22.0 [3] ± 0.30	21.1 [3] ± 0.29	48.8 [3] ± 0.55	46.7 [3] ± 0.52
Fat	1st	108	22.0	21.5	47.6	46.5
	2nd	120	19.8	19.2	44.1	42.7
	3rd	216	19.8	19.2	43.4	42.4
	Total	444				
	Average		20.3 ± 0.29	19.8 ± 0.32	44.6 ± 0.45	43.5 ± 0.48

[1] All litters reduced at three days of age to 6 young, usually three of each sex.

[2] Standard error of the mean.

[3] Significantly different at 1% level from either control or fat group.

fective in producing heavier weanling animals. A similar beneficial effect of sucrose was observed by Meyer et al. ('51).

It is interesting to note that the high-fat diet was the equal of the control diet in promoting lactation, in contrast to its limiting effect on reproduction. Further, the improvement in lactation resulting from the high-carbohydrate diet as compared to the control diet was not accompanied by any beneficial effect on reproduction. These observations support the thesis of Sica and Cerecedo ('48) that the reproductive process is the more critical of the two.

All dams were successful in rearing the 6 young given them to nurse with the exception of one on the fat diet that lost one of her young between the 14th and 21st days of lactation.

The average change in weight of the dams during the lactation period from the third to the 14th day was + 7.0, + 9.4 and — 4.2 gm, respectively, for the control, carbohydrate and fat diets, and from the 14th to 21st day these values were — 10.0, — 8.0 and — 2.2 gm. The individual variation on all diets was extreme, however, ranging from + 49 to — 34 gm. A more severe loss in weight of dams receiving high-fat diets during the first 17 days of lactation was reported by Maynard and Rasmussen ('42) in paired feeding studies involving isocaloric intakes at different fat levels of diets composed of either natural or purified components. The weight loss was reversed, however, when the high-fat, purified diet was fed ad libitum. There is some question whether this result would have been obtained if all factors necessary for lactation had been provided in optimum amounts or if the diet which contained 18% fat had consisted of natural foods. Purified diets containing up to 40% of fat have been shown by Deuel et al. ('47) to permit excellent reproduction and lactation.

Maynard and Rasmussen also found a fat level of 9% superior to one of 4.5% in natural food diets, whereas in the present experiment a diet containing 23% of fat was just equal to the control diet (4.4% fat) and was less efficient than the high-carbohydrate diet (3.4% fat) in promoting lac-

tation. Apparently the optimum level of fat may be greatly influenced by the composition (purified or natural food components) of the diet.

SUMMARY

Reproduction and lactation performance was measured for three generations with albino rats fed diets ad libitum composed essentially of natural foods and varying in fat and carbohydrate content.

With an average number of 78 matings for each diet, a significant decrease in reproductive performance of rats receiving the 23% fat diet was observed when compared to the control (4.4% fat) and the high-carbohydrate-fed (3.4%) animals. This was manifest in smaller numbers of lighter weight young in the litters of the high-fat group.

Lactation performance, measured with an average number of 350 nursing young for each diet, revealed no impairment due to the high-fat diet as compared to the control diet. However, a significant improvement in lactation resulted from the high-carbohydrate diet which contained sucrose. Weanling weights and weight gains of the young during the critical lactation period were increased 7 to 10% in this group.

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NITROGEN CONSTITUENTS OF SOW'S MILK AS AFFECTED BY RATION AND STAGE OF LACTATION [1]

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The literature reveals very little information on the detailed composition of sow's milk. The main reasons for this lack of information are the difficulties involved in obtaining representative samples in large enough quantities for analysis. Various workers have reported studies on gross composition. The majority of the reports were not concerned with the effects of either ration or stage of lactation, but merely with composition per se. This, to a large extent, helps to explain the wide disparity in the reported data.

More recently Braude et al. ('46, '47) reported on the gross composition of sow colostrum and normal milk, their work including analyses for several vitamins. Bowland et al. ('49a, b, c) studied the effect of nutrition on the gross composition of sow's milk, comparing stage of lactation and dry-lot versus pasture feeding primarily with respect to content of vitamins A and C. The studies of both Braude and Bowland were conducted under controlled conditions.

None of the aforementioned studies includes data on the various nitrogenous components of sow's milk.

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EXPERIMENTAL PROCEDURE

The work reported herein will be discussed in 4 different sections.

Section I

Twenty-five sows, 8 Chester Whites and 17 Poland Chinas, were distributed at random into 5 lots. From weaning until two months before breeding these sows were fed a ration of corn, soybean oil meal, and 5% alfalfa. During the course

TABLE 1

Ration of sows in section I [1]

INGREDIENTS OF RATION	LOT				
	I	II	III	IV	V
	lb.	*lb.*	*lb.*	*lb.*	*lb.*
Yellow corn	79.0	80.0	81.0	83.5	79.0
Soybean oil meal	14.5	8.5	7.5	..	14.5
Ground alfalfa	5.0	5.0	5.0	5.0	5.0
$CaCO_3$	1.0	1.0	1.0	1.0	1.0
Iodized salt	0.5	0.5	0.5	0.5	0.5
Fish solubles	..	5.0	..	..	..
Tankage	..	..	5.0	10.0	..
B_{12} concentrate	..	..	..	..	0.5

[1] All rations adjusted to an equivalent total protein content (14%).

of the experiment the sows were fed as indicated in table 1. Samples of colostrum,[3] 15-, and 30-day milk were analyzed for total and casein nitrogen. The milk was extracted manually after injection of 1 ml of Pitocin [4] into the ear vein of the sow. Two men, one on each side, milked simultaneously as rapidly as possible in order to complete the milking by 6 minutes after the oxytocin injection. Four hundred to 600 gm per milking were obtained. All teats were milked. The results are summarized in tables 2 and 3.

[3] In this work colostrum is milk extracted 12 to 24 hours after parturition.
[4] Parke-Davis.

TABLE 2

Effect of ration on the distribution of the nitrogen of colostrum, 15-, and 30-day milk of sows in section I

ITEMS COMPARED	LOT I	LOT II	LOT III	LOT IV	LOT V
			Total nitrogen (mg per 100 gm)		
Colostrum	1,354 ± 94.4 [1]	1,501 ± 131.2	1,827 ± 209.6	1,720 ± 99.2	1,694 ± 224.0
15-day milk	..[2]	824 ± 44.8	757 ± 43.2	763 ± 24.0	819 ± 8.0
30-day milk	..[2]	994 ± 116.8	869 ± 52.8	768 ± 25.6	813 ± 22.4
			Casein nitrogen (mg per 100 gm)		
Colostrum	671 ± 75.2	678 ± 17.6	611 ± 36.8	752 ± 28.8	725 ± 84.8
15-day milk	..[2]	533 ± 17.6	550 ± 44.8	546 ± 69.6	557 ± 56.0
30-day milk	..[2]	605 ± 24.8	546 ± 11.2	544 ± 68.8	558 ± 19.2

[1] Standard error of the mean.
[2] All of the baby pigs died and the sows ceased lactating before the 15th day.

TABLE 3

Effect of stage of lactation on the distribution of the nitrogen of colostrum, 15-, and 30-day milk of sows in section I

ITEMS COMPARED	COLOSTRUM	15-DAY MILK	30-DAY MILK
Total nitrogen (mg/100 gm)	1646 [1] ± 76.8 [2]	808 ± 27.2	862 ± 40.0
Total protein (%)	10.50 ± 0.49	5.15 ± 0.17	5.50 ± 0.26
Casein nitrogen (mg/100 gm)	683 [1] ± 24.0 (41.5) [3]	554 ± 28.8 (68.6) [3]	565 ± 28.8 (65.5) [3]
Casein protein (%)	4.35 ± 0.15	3.53 ± 0.18	3.60 ± 0.29
Other nitrogen (mg/100 gm)	963 [1]	254	298
Other protein (%)	6.15 (58.5) [3]	1.59 (31.4) [3]	1.90 (34.5) [3]

[1] Significant at 1% level.
[2] Standard error of the mean.
[3] Percentage of the total protein.

Section II

Three sows selected at random from section I in their 7th week of lactation were milked. Sow 2 came from lot V and the other two sows were from lot IV. Their milks were analyzed in great detail, with special emphasis on the nitrogenous fractions. The results are tabulated in table 4.

TABLE 4

Detailed analysis of sow's milk

(mg per 100 gm milk)

N-FRACTION	SOW			Average
	2	4	7	
Total N	1,126	992	880	999
Casein N	693	545	477	572
Albumin N	93.1	62.4	67.5	74.3
Globulin N	97.5	112.5	95.8	101.9
Proteoses-peptone N	164	188	175	176
N.P.N.	78.3	84.0	64.8	75.7
Ammonia N	0.80	1.75	0.95	1.16
Urea N	12.3	13.2	13.7	13.1
Creatinine[1]	1.90	1.78	1.80	1.83
Creatine[1]	9.65	10.8	7.40	9.28
Uric acid[1]	2.84	2.20	2.03	2.36
Alpha-amino N	27.8	18.8	22.2	22.9
N unaccounted for	32.6	45.3	24.2	34.0
Total solids, %	21.0	19.2	17.4	19.2
Fat, %	8.31	7.46	5.62	7.13
Lactose, %	5.07	6.76	5.31	5.71
Sulfur, mg %	74.2	73.3	63.7	67.1
pH	7.00	7.07	6.90	6.99
Specific gravity	1.036	1.040	1.039	1.038
Freezing point, °C.	— 0.562	— 0.564	— 0.564	— 0.563

[1] Reported as such. N unaccounted for calculated on the basis of N only.

Section III

Twelve grade Hampshire sows, 6 between the 4th and 15th day of lactation and 6 between the 15th and 38th day of lactation, were milked, and analyses for total, casein, and nonprotein nitrogen were made. Table 5, part A, presents a summary of these data. These sows were on excellent ladino-

alfalfa pasture during the gestation period. During the last half of the gestation period, in addition to pasture, they were hand-fed first one and then two ears of corn per sow per day. Seven days prior to farrowing they were taken to the farrowing barn and self-fed the following ration: 15 parts alfalfa, 15 parts of a 40% protein commercial hog supplement, 5 parts of corn, 15 parts of wheat, 48 parts of oats, and one part each of $CaCO_3$ and trace mineralized salt (16.3% total protein).

Section IV

Four purebred Poland China and 6 purebred Chester White sows were milked on the first, 15th, and 30th day. The samples were frozen and analyzed for non-protein nitrogen three months later. Prior to farrowing these sows were on good alfalfa-brome-ladino pasture and were self-fed soft ear corn in addition to a mixture consisting of 46 parts ground corn, 30 parts oats, 17 parts wheat middlings, three parts linseed meal, three parts tankage, and a half part each of calcium carbonate and trace mineralized salt (13.3% total protein). Three days before farrowing the sows were brought into the farrowing barn and hand-fed the following mixture: 20 parts ground corn, 20 parts oats, 20 parts wheat middlings, 15 parts wheat bran, 8 parts linseed meal, 4 parts tankage, one-half part calcium carbonate, one-half part trace mineralized salt, and 12 parts alfalfa leaf meal (17.3% total protein). The sows were fed a limited quantity for a few days after farrowing and then full-fed the above mixture. The summary of this study appears in part B of table 5.

All the methods for determining protein and non-protein fractions standardized for cow's milk (Shahani and Sommer, '51) were applicable to sow's milk, except that smaller aliquots were taken for the total, non-protein, globulin, and alpha-amino nitrogen determinations. Total solids were determined by drying a weighed sample of milk in a vacuum drying oven, at 70°C. and 5 mm vacuum. Fat was determined by the Mojonnier technique, lactose by the Munson

and Walker copper reduction method, and sulfur by reducing sulfur to hydrogen sulfide, which was estimated iodometrically. The Beckman pH meter was used for pH determinations; the Quevenne lactometer, for specific gravity; and the Hortvet cryoscope, for the freezing point determinations.

TABLE 5

Lactation and nitrogenous constituents of milk

Part A. Effect of stage of lactation

ITEMS COMPARED	4–15 DAY	15–38 DAY
Total nitrogen (mg/100 gm)	780 ± 34.2	808 ± 45.5
Total protein (%)	4.98 ± 0.22	5.16 ± 0.29
Casein nitrogen (mg/100 gm)	495 ± 24.5	506 ± 21.9
Casein protein (%)	3.16 ± 0.16 (63.5)[1]	3.23 ± 0.14 (62.6)[1]
Non-protein nitrogen (mg/100 gm)	83.1 ± 8.5	95.4 ± 10.4
(N × 6.38) % protein	0.53 ± 0.05 (10.6)[1]	0.61 ± 0.07 (11.8)[1]
Other nitrogen	202	207
(N × 6.38) % protein	1.29 (25.9)[1]	1.32 (25.6)[1]

Part B. Effect of lactation on N.P.N. of sow's milk

ITEMS COMPARED	1-DAY	15-DAY	30-DAY
Non-protein nitrogen (mg/100 gm)	70.8 ± 3.9	131 ± 8.1[2]	137 ± 6.8[2]
Expressed as protein (%)	0.45 ± 0.02	0.84 ± 0.05	0.88 ± 0.04

[1] Percentage of the total protein.

[2] Significant at 1% level.

RESULTS

Section I

The object of this experiment was to determine the effect of vitamin B_{12} and animal proteins upon the various nitrogenous constituents of the milk of sows fed a corn, soybean oil meal, 5% alfalfa (C.S.A.) ration. This ration has been shown by Rose et al. ('44) to cause failure in both reproduction and lactation. It has been suggested that the milk of these sows may lack certain qualities possessed by the milk of sows fed rations supplemented with animal proteins.

Since vitamin B_{12} has been associated with the metabolism of protein (Charkey, Wilgus, Patton and Gassner, '50; Hart-

man, Dryden and Cary, '49; Henry and Kon, '51), one would look for differences in the nitrogenous components of milk from sows on C.S.A. rations. Although considerable variation within lots was observed, colostrum, 15-, and 30-day milk from sows in all groups had a similar total nitrogen and casein nitrogen content (table 2). The total nitrogen values for the colostrum of the sows in lot I tended to be lower than those for the colostrum of sows fed B_{12} or animal protein, but these differences were not significant (F test). The total nitrogen content of 15- and 30-day milk was not significantly different in the B_{12}- or animal protein-supplemented lots. The casein nitrogen tended to be less variable than the total nitrogen content.

Because there were no significant differences between treatments, it was decided to pool all the data in evaluating the effect of stage of lactation (table 3). The total protein content of the 15th day milk was less than half that for colostrum. There was a tendency for the total protein to rise slightly from the 15th day milk to that of the 30th day. This is in general agreement with the work of Bowland et al. ('49c) insofar as the trend is concerned. The levels of total protein, however, were consistently lower, and were similar to those of Braude et al. ('47).

The casein fraction was quite similar in concentration pattern to that for total protein, but the changes were not as marked. No consistent breed difference was observed.

It is of interest to note that the casein in normal sow's milk makes up only 67% of the total protein (colostrum, 41.5%), in contrast to an average of 76% in normal cow's milk (Davies, '33).

Failure to observe a difference in total or casein protein (table 2) is not surprising, since it is generally known that when the lactating animal is forced to conserve milk, because of a scarcity of structural units, it lowers the flow, but at the same time tends to maintain the milk's normal composition. Thus, with the exceptions of fat (Willett and Maruyama, '46), vitamins, and the various trace minerals, the or-

ganic and inorganic constituents of milk remain relatively constant under wide variations of diet. Parrish et al. ('48), feeding high and low protein rations to dairy cows, reported that the rations did not effect significant differences in the levels of the total protein, casein, and albumin-globulin fractions of colostrum or early milk. Riddet et al. ('41) were able to show a definite decrease in the non-fat-solids of cow's milk when cows were under conditions of subnormal feeding. This was ascribed to a decrease of approximately 0.2% in total protein and an approximate 0.1% decrease in lactose content. Regan and Richardson ('38) made a similar observation, but only after subjecting the cows to high atmospheric temperatures.

Section II

Sow's milk was found to be richer, greater in specific gravity, more alkaline in nature, and to have a slightly lower freezing point than cow's milk.

The data are in harmony with the values reported by Braude et al. ('47) except in the case of lactose. As compared to the figures cited by Jackson ('50), the values obtained in this study were higher in total solids, fat, and lactose, but lower in total proteins. A detailed analysis of the various nitrogen fractions appears in table 4. Casein accounted for 57 to 58%, albumin 7 to 8%, globulin 10%, proteoses-peptones 17 to 18%, and non-protein nitrogen 7 to 8% of the total nitrogen. Among non-protein nitrogen fractions, alpha-amino and urea nitrogen fractions were highest and both together accounted for about 85% of the measurable non-protein nitrogen. As compared to cow's milk, sow's milk was about 99% higher in total nitrogen (considering 500 mg total nitrogen per 100 ml cow's milk as average). There also was a pronounced difference between the albumin and globulin content of sow's milk and that of cow's milk. The former contained larger amounts of albumin and globulin, and the amount of globulin was larger than that of albumin, whereas in the case of cow's milk the quantity of albumin nitrogen

is greater. A similar globulin-albumin relationship exists in the plasma proteins of swine as was found in the milk.

The non-protein nitrogen content of sow's milk is significantly higher than that of cow's milk. The average non-protein nitrogen content of the three sow's milk samples was about 2.3 to 2.4 times the average for cow's milk (Shahani and Sommer, '51). Block and Bolling ('50) reported that human breast milk contained 4 times the non-protein nitrogen of cow's milk. In terms of non-protein nitrogen fractions, these higher values for sow's milk were due chiefly to the higher values found for urea, alpha-amino nitrogen, and nitrogen unaccounted for.

Section III

The same trend, a tendency for the various nitrogenous constituents of normal milk to increase with stage of lactation, was in evidence in this group of pasture-fed sows (table 5, part A). The observation by Bowland et al. ('49c) that the total protein of milk from pasture-fed sows was consistently lower than that of dry-lot sows, although the difference was not statistically significant, was borne out by this study. This work showed that these differences were due primarily to the lower level of casein in the milk of pasture-fed sow. The nitrogen other than casein was actually slightly higher in pasture-fed sows than in dry-lot sows, which was probably due to the non-protein nitrogen. One cannot say with any certainty that these differences were due to the effects of pasture versus dry-lot feeding. Although no apparent breed difference was observed in the section I study or in the work of Bowland et al. ('49c), one can never completely rule it out, particularly under slightly different interacting conditions.

Section IV

The non-protein nitrogen level of colostrum was found to be 70.8 $\pm$ 3.9 mg per 100 gm of sample, rising to almost double that value by the 15th day, with a further slight in-

crease in 30-day milk (table 5, part B). These increases over the values for colostrum were highly significant.

In studies with dairy cattle Parrish et al. ('48) were able to show that a high protein ration raised not only the non-protein nitrogen content of colostrum and normal milk but also that of blood serum, in contrast to the effect of a low protein ration. As lactation progressed there was a gradual decrease in the amount of non-protein nitrogen in the milk of both the high and low protein lots.

Perkins et al. ('32) concluded that the differences that were found in the protein composition of cow's milk as a result of changing the amount or quality of protein in the ration were principally reflected in the non-protein nitrogen portion of the milk.

In general the work reported here was characterized by the existence of considerable individual variability among sows regardless of ration or stage of lactation.

SUMMARY AND CONCLUSIONS

The effect of ration and stage of lactation on total protein, casein, and non-protein nitrogen in the milk of the sow was studied. A detailed analysis of sow's milk with emphasis on the nitrogenous constituents was made.

The additions of B_{12}, tankage or fish solubles to an all-plant protein ration did not result in significant changes in the total protein or casein content of the colostrum of sows previously maintained on a corn, soybean oil meal, 5% alfalfa (C.S.A.) ration, when the total protein of the ration was the same. Similarly, the source of protein had no effect on the amount of total or casein protein in the 15- and 30-day milk of sows in lots II, III, IV, and V.

The total protein content dropped sharply from colostrum to 15-day milk, but rose slightly in 30-day milk. The casein content values behaved similarly but the changes were not as marked. The decrease from colostrum to 15-day milk was highly significant in both instances.

The non-protein nitrogen increased significantly from colostrum to 15-day milk and there was a further increase in 30-day milk.

The mean values of sow's milk constituents (7th week of lactation) were as follows: 19.2% total solids, 7.13% fat, 5.7% lactose, 6.4% protein (N × 6.38), sulfur 67.1 mg %, pH 6.99, specific gravity 1.038, and freezing point —0.563°C. Of the 6.4% protein, casein accounted for 57 to 58%, albumin 7.8%, globulin 10%, proteoses-peptones 17 to 18%, and non-protein nitrogen 7 to 8%. Alpha amino and urea nitrogen fractions made up about 85% of the accounted-for non-protein nitrogen.

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PROCEEDINGS OF THE SIXTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

HOTEL NEW YORKER, NEW YORK CITY
APRIL 14–18, 1952

COUNCIL MEETINGS

Council Meetings were held in the Hotel New Yorker on Sunday, April 13, and Monday, April 14. Formal actions of the Council are reported in the following minutes of the business meetings.

SCIENTIFIC SESSIONS

The scientific program of the Institute consisted of 7 half-day sessions at which 80 submitted papers were presented and one half-day session devoted to a symposium on "Geriatric Nutrition." The symposium was held on Wednesday afternoon, April 16. Four papers were read by title.

BUSINESS MEETINGS

Two business meetings were held: one at 4:00 P.M. Tuesday, April 15, and one at 4:00 P.M. Thursday, April 17. The following items were considered:

The Tuesday business meeting. The meeting was called to order by President Clive M. McCay. The minutes of last year's business meetings, as published in the August, 1951, issue of the *Journal of Nutrition,* were approved.

The President appointed Dr. R. A. Gortner, Jr. and Dr. Charlotte Roderuck to serve as a Tellers' Committee for the election of officers. The ballots were transmitted to the Tellers' Committee by the Secretary.

The Council's recommendations on nominees for election to membership in the American Institute of Nutrition were

presented. It was voted to approve the Council's recommendations. The following were elected to membership:

Joseph T. Anderson
W. M. Beeson
Bertha S. Burke
Jack M. Cooperman
Theodore S. Friedemann
Marvin B. Gillis
Albert C. Groschke
Arthur M. Hartman
Carl M. Lyman
Charles D. May
James McGinnis
Joseph Meites
Robert E. Olson
Howerde E. Sauberlich
Bureh H. Schneider
Max O. Schultze
Jakob A. Stekol
Norman C. Wetzel
Hilda F. Wiese
C. K. Whitehair

The report of the Treasurer, Dr. N. B. Guerrant, was submitted. The Auditing Committee, consisting of Dr. R. W. Swift and Dr. Alex Black, reported that receipts and disbursements are properly recorded in the Treasurer's books and that his report as of April 1, 1952, is substantiated by the record.

The report of Dr. W. C. Russell, representative of the American Institute of Nutrition to the Division of Biology and Agriculture and to the Food and Nutrition Board, National Research Council, was submitted.

The representatives on the Joint Committee on Nomenclature, Dr. C. A. Elvehjem and Dr. E. M. Nelson, reported no actions during the year.

The annual report of the Editor of the *Journal of Nutrition* was presented by Dr. George R. Cowgill. The report follows.

Annual Report of the Editor of the Journal of Nutrition

This annual report covers the volumes of the Journal of Nutrition published during 1951 as well as general matters arising since the last annual meeting of the American Institute of Nutrition in Cleveland, Ohio, April 15, 1951.

Papers published and related data

Three volumes — numbers 43, 44 and 45 — were published during 1951. Each volume began with a biography. In addition, these three volumes contained 45, 47 and 49 articles, respectively, giving a total

of 144 for the year, compared with 151 for the previous year. The average number of pages per article proved to be 12.5, exactly the same as for 1950. The present arrangement with our publisher, Wistar Institute, calls for three volumes a year and the printing of 1,800 pages of scientific matter. During 1951 subscribers received a total of 1,941 pages; during the previous year 1,895 pages were printed. During the year 197 papers were submitted, of which 39, or 19.8%, were rejected. The corresponding figures for 1950 are 170 submitted and 43, or 25.3%, rejected.

Picture on front cover

In continuation of the policy adopted at the 1949 meeting of the editorial board in Detroit, the first issue of each volume carried a new picture in the oval on the front cover. A larger picture was used as a frontispiece, accompanied by a short biography. The pictures used in 1951 were those of Magendie, Beaumont and Bernard.

Personnel of editorial board

At the last annual meeting three members retired, having served their terms of 4 years. They were E. W. Crampton, O. L. Kline, and R. W. Swift. The new members elected to replace them were Alex Black, Floyd S. Daft, and Harry G. Day. For part of the year Dr. Hegsted was absent on professional work in Peru. He offered to resign because of this enforced absence but was prevailed upon to consider himself as being on a "leave of absence." On his return in June, 1951, he resumed active work as a member of the board.

Editorial problems

In my last annual report I commented rather extensively on these problems and therefore write about them only briefly here. They continue to be the same as those encountered in previous years. As part of our effort to deal properly with them I try to have an annual meeting of the editorial board at each Federation meeting. At such times editorial problems are reviewed, an exchange of ideas obtained, the actions of the editor reviewed, and any matter pertaining to the Journal and its operation considered. This is mentioned here in order to make clear to the members of the Institute that the editor tries not to be dictatorial in the discharge of his responsibilities, actively seeks the advice of the editorial board in the determination of policy, and endeavors to keep the board properly informed and in a position to render real service in the printing enterprise represented by the Journal.

The editorial office thinks it detects *some* improvement in the matter of authors' reading the instructions to contributors printed on the inside back cover of each issue. It is still evident, however, that many authors do *not* read such instructions.

General comments

It is appropriate to express here my deep appreciation of the services rendered by the individual members of the editorial board. Our thanks are also due to the staff of The Wistar Institute, which has always cooperated in solving special printing problems as far as possible in accordance with our wishes. Some issues have been delayed because of factors beyond their control. Examination of any issue with respect to the dates when papers were submitted for publication will reveal what appears on the surface to be an inexcusable delay in final publication. Examination of our office records in this connection, however, will show that such delay was not our fault but really due to the author's failing to give reasonably prompt attention to the revision of his manuscript. We have actually had some papers that were returned for revision sent back to us after as long as a year! A check shows that, *on an average*, an accepted paper appears in about 4½ or 5 months after formal submission. In a few instances there have been long delays for which we were responsible and for which we offered our apologies. Fortunately, our records show such instances to be fewer and fewer. I wish we could avoid them entirely, but suppose that this represents an unattainable ideal.

A motion was passed expressing appreciation to Dr. Cowgill for his excellent administration of the affairs of the Journal.

President McCay reported on the actions of the Federation Executive Committee during the past year. Attention was directed to the activities of the "Animal Welfare Institute" in attempting to restrict or prevent animal experimentation in certain parts of the country. The Federation Executive Committee regards these activities as a serious menace to research in the biological sciences and is taking steps accordingly.

The proposed new Federation constitution and by-laws were discussed. Mimeographed copies were distributed to all members present for study. The Secretary stated that the proposed new constitution and by-laws embody the modifications approved at the business meeting last year. A few

other changes of less importance are also included. The document has been approved by the Federation Executive Committee after several months' careful study and the Council of the Institute. A motion to approve the new Federation constitution and by-laws was carried unanimously.

The meeting was adjourned at 5:10 P.M.

The Thursday business meeting. The meeting was called to order by President McCay at 4:00 P.M. At President McCay's request, an extension for another year of the changes in the Federation by-laws made last year at the Cleveland meeting was approved. Such an extension will become necessary only if the new Federation constitution and by-laws are not approved by the other societies of the Federation.

A motion to include the past-president as a member of the Council of the Institute for the coming year was approved. This change was suggested because the past-president of each Society is now a member of the Federation Executive Committee. A corresponding change in the constitution and by-laws of the Institute will be submitted for approval next year.

Dr. Pollack reported on the work of the Committee on the Registry of Pathology of Nutritional Diseases. A summary of the work of the Registry is to be published in the *Journal of Nutrition.*

Dr. W. H. Griffith discussed the organization of the American Board of Nutrition and its activities during the past year.

The Teller's Committee reported the results of their count of the ballots. A total of 230 ballots was cast. The following officers were elected for the year beginning July 1, 1952:

President:
Paul L. Day

Vice-President:
Conrad A. Elvehjem

Councillor:
Gladys A. Emerson

Associate Editors:
James B. Allison
Carl A. Baumann
L. C. Norris

Suggestions for the Nominating Committee:
The names of 16 members receiving 10 or more votes each were received.

A motion was passed to set the annual dues at $1.00 per year, as recommended by the Treasurer and approved by the Council.

As approved by the Council, the recommendation that the annual $3.00 Federation assessment per member be paid, if desired, by retired members was unanimously adopted. As is the case in other societies of the Federation, retired members of the Institute now may or may not elect to pay the assessment and receive in return the *Federation Proceedings*. In either event, they will continue to be listed in the Federation Directory. Heretofore, the assessment has been paid from the treasury of the Institute. It was pointed out that this practice could no longer be continued without an increase in Society dues, because of the increasing number of emeritus members.

The desirability of re-establishing a Committee on Food Habits and Methods of Education by the National Research Council, either as a unit or with the Food and Nutrition Board, was discussed by Dr. P. E. Howe. By motion, the members approved the establishment of such a committee and the aiding of the project in any way possible.

President McCay announced that the members of the American Institute of Nutrition have been invited to participate in the 19th International Physiological Congress to be held in Montreal, August 31 to September 4, 1953. Notices regarding the meetings will be mailed to members at a later date.

Dr. E. M. Nelson spoke briefly on the progress of the organization of the International Union of Nutritional Sciences.

President McCay announced that next year's meetings will be held in Chicago. In 1954 they will be held in Atlantic City and, tentatively, in Los Angeles in 1955. The Federation Executive Committee has requested each Society secretary to poll the membership as to preference for meeting *annually* in Atlantic City after 1955.

The meeting was turned over to the new President, Dr. Paul L. Day, and adjourned at 5:15 P.M.

THE ANNUAL DINNER AND PRESENTATION OF AWARDS

The Annual Dinner of the Institute of Nutrition was held on Wednesday evening, April 16, in the Hotel New Yorker. The program consisted of the introduction of new members, the presentation of awards, and a few informal remarks by President McCay.

The Borden Award was presented to Dr. Max Kleiber of the University of California at Davis for his contributions regarding factors involved in the formation and utilization of the constituents of cow's milk. The achievements of the medalist were described by Dr. Walter C. Russell of Rutgers University. Dr. Kleiber responded with a brief resumé of the work of his group.

The Mead Johnson Vitamin B-Complex Award was presented to Dr. Howerde E. Sauberlich of the Alabama Polytechnic Institute in recognition of his fundamental investigations on the citrovorum factor and its relation to folic acid. Dr. W. D. Salmon, of the same institution, introduced the Award recipient and commented on his contributions. Dr. Sauberlich then spoke humorously regarding his investigations.

The Osborne and Mendel Award was presented to Dr. Icie Macy Hoobler, Scientific Director of the Children's Fund of Michigan, for her outstanding studies on the nutritive requirements of children. Dr. Arthur H. Smith of Wayne University, in introducing the medalist, spoke of her early training and her many subsequent achievements in nutritional research on children. Dr. Hoobler responded with reminiscences of her studies and with a tribute to the inspiration and guidance which gave direction to her work by her teacher, for whom the award was jointly named, Professor Lafayette B. Mendel.

COMMITTEES FOR 1952–1953

President Clive M. McCay appointed the following committees for the year beginning July 1, 1952:

Nominating Committee

J. H. Roe, Chairman
R. J. Block
L. R. Cerecedo
W. D. Salmon
Pearl P. Swanson

Committee on Registry of Pathology of Nutritional Diseases

H. Pollack, Chairman
O. A. Bessey
W. H. Sebrell, Jr.
E. L. Sevringhaus

Representatives to the Joint Committee on Nomenclature

C. A. Elvehjem
E. M. Nelson

Representative to the Division of Biology and Agriculture and to the Food and Nutrition Board, National Research Council

W. C. Russell

Respectfully submitted,
JAMES M. ORTEN, *Secretary*
American Institute of Nutrition

BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1953 Award and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the Jury of Award may recommend that it will be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1953. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1953. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

Chairman, Nominating Committee:

DR. LEO T. SAMUELS
Department of Biological Chemistry
University of Utah Medical School
Salt Lake City, Utah

OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1953 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1953.

Chairman, Nominating Committee:

DR. PHILIP HANDLER
Department of Biochemistry and Nutrition
Duke University School of Medicine
Durham, North Carolina